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# Addition of Saturated and Trans-fatty Acids to the Diet Induces Depressive and Anxiety-like Behaviors in *Drosophila melanogaster*

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**Abstract—This study aimed to evaluate the effects of the addition of saturated fat and hydrogenated vegetable fat (HVF) to the diet on depressive and anxiety-like behaviors in *Drosophila melanogaster.* Flies were exposed to experimental diets: regular diet (RD), or HVF in the concentrations of the substitute (SHVF), HVF 10% and HVF 20%, or Lard (L) in the concentrations of the substitute (SL), L 10% and L 20%, during seven days. Our results showed that flies fed with the HVF diet presented similar behaviors to depression, anxiety, and a higher number of aggressive events. Flies exposed to L showed only depressive-like behavior. Regarding serotonin levels (5HT), there was a significant reduction in the flies exposed to SHVF, HVF 10%, HVF 20%, and L 20%. Regarding the levels of octopamine (OA), there was a significant reduction in the flies exposed to both HVF and L rich diets when compared with the RD group. Also, there was a significant negative correlation between 5HT or OA levels and behaviors of aggressiveness, negative geotaxis, immobility time, light/dark, and grooming in the flies. This study shows that *D. melanogaster* can serve as a valuable model for understanding psychiatric disorders and that the type of fatty acid (FA) offered in the diet can influence these disorders. This demonstrates the importance of the composition of the FAs in the neural pathways, being able to influence the signaling of neurotransmitters, such as 5HT and OA, and thus, cause behavioral changes.** 

**Key words: metabolism, cell membrane, dietary composition, psychiatric disorders, serotonin, neurotransmission.**

## INTRODUCTION

Food consumption is essential because it's the main source of energy demand, and it's related to the homeostasis of the organism. However, any imbalance in the caloric intake can cause negative consequences for the metabolism. The world population has a high consumption of industrialized food (Lam and Adams, 2017). These go through a type of processing with the intent of increasing taste, palatability, consistency, odor, and durability, among others, so it becomes more pleasant for the consumer. However, these foods can be considered a substantial threat to health since many tend to have a higher content of saturated and trans fat (Adams and White, 2015).

The high consumption of saturated fat is associated with the manifestation of cardiovascular diseases, due to the increase of the LDL cholesterol concentration in the blood (Siri-Tarino et al., 2010). For this reason, food industries had to obtain a way to reduce the amount of saturated fat in food, consequently decreasing their total fat content, thus changing the formulation of these products, where the saturated FA were replaced per trans FA (Liss and Marangoni, 2017). The trans FA is defined by geometric isomers of monounsaturated and polyunsaturated FA (PUFA) with at least a double bond in the trans configuration. This type of FA has always been part of the human diet since it's natural form is present in products of animal origin, but, due to frequent modifications in the feeding patterns and industrial advances, there was an

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*Abbreviations:* 5HT, Serotonin; FA, fatty acid; HVF, hydrogenated vegetable fat; L, Lard; OA, Octopamine; PUFA, polyunsaturated fatty acid; RD, regular diet; SHVF, hydrogenated vegetable fat substitute; SL, Lard substitute.

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increase in the use and consumption of this type of fat (Costa et al., 2006; Iqbal, 2014). However, as well as saturated fat, trans fat also harms health. The hydrogenation process of FA provokes the loss of metabolic activity of the natural FA, and among the effects of that metabolic alteration, one of the most dangerous is the increased risk of cardiovascular diseases (Costa et al., 2006; Semma, 2002).

Furthermore, trans type of fatty acid is associated with the development of neurological problems. This type of fat can end up incorporating in the neural lipid bilayer, causing changes in neurotransmission, thus increasing vulnerability to psychiatric diseases, such as anxiety and depression. These psychiatric disorders are responsible for affecting millions of people globally, and it can even result in death (Haygert et al., 2018; Sharma et al., 2012). According to the OMS in the next years, these disorders may reach the second place of the leading causes of diseases in the world, with depression being the type of illness considered more incapacitating.

Thus, understanding the mechanism of such human disease is extremely important. It can make it easier to search for alternative treatments, which aim to reduce the damage caused by such disorders. Usually, rodents' models are widely used to understand psychiatric disorders (Campos et al., 2013). However, access to these animals is becoming increasingly limited. On this, it is of great importance to search for alternative experimental models that are effective and can discuss human physiology, besides easy access and maintenance, and that enable the understanding of diseases mechanisms in humans, from metabolic changes, to even neurological disorders, so that later the experimental techniques become more viable and thus, the search for alternative treatments (Morales, 2008).

On this, a model that has been bringing promising results related to the understanding of human diseases is the *Drosophila melanogaster*. This model and humans share many genes, conserve metabolic and signaling pathways, and, besides that, there is growing evidence of conservation at behavior level and of their molecular mechanisms, including circadian rhythms, learning, memory, and sleeping (Benton, 2008; Pandey and Nichols, 2011). Besides that, it is a useful model for studies of lipid metabolism and energy homeostasis, since in insects, as well as in vertebrates, lipids play essential roles in the organism (Atella et al., 2012; Liu and Huang, 2013). Besides constituting the neural membrane, they are responsible for characteristics such as permeability, thickness, and fluidity. Thus, as in vertebrates, the type of FA present in the diet is extremely important for the proper functioning of the fly's nervous system, where an inadequate intake of FA can lead to changes in neurotransmitters signaling and consequently increase the susceptibility to neurological diseases, such as depression (Vrablik and Watts, 2013; Ziegler et al., 2015). Here we show that the type of FA coming from the diet may cause changes in monoamines, being correlated with changes in behaviors similar to depression and anxiety. It can also cause an increase in aggressive behavior in *D. melanogaster*.

## EXPERIMENTAL PROCEDURES

### Materials and fly culture conditions

The HVF (rich in trans FA) was produced by Coamo® (Paraná, Brazil) and purchased in the free market. The L (rich in saturated FA) was produced by Alibem® (Santa Rosa, Brazil) and purchased from a local supermarket. The Sodium Dodecyl Sulfate (SDS; Cat. L3771), Sucrose (S5016), and Hepes Minimum 99.5% (Titration, H3375) were acquired from Sigma-Aldrich® (São Paulo, SP, Brazil).

*D. melanogaster* (Harwich strain) were obtained from CIPBIOTEC (Unipampa/São Gabriel). The flies were maintained in BOD incubators, with a light photoperiod of 12 h (25 °C ± 1 °C; 60 % of humidity), fed with the standard laboratory food which contains: cornflour (76.59%), sugar (7.23%), wheat germ (8.51%), salt (0.43%), powdered milk (7.23%) and Nipagin (0.08%, an antifungal agent). The progenitor flies were maintained in this environment until mating and egg-laying. Afterward, the adults were removed, and the hatched flies were used in the treatments (approximately three days old).

### Fat and diet composition

The fat content of the diets was determined by the gravimetric method of Bligh and Dyer (1959), and it was used to determine the FA composition of the diets. The fats were saponified in a methanolic Potassium hydroxide solution and esterified in a methanolic sulfuric acid solution (Hartman and Lago, 1973). Methyl ester FA was analyzed using an Agilent Technologies gas chromatograph (HP 6890) equipped with a DB-23 capillary column (60 m × 0.25 mm × 0.25 μm) and a flame ionization detector. The injector port temperature was set at 250 °C, and the carrier gas was nitrogen (0.6 mL/minute). After the injection (1 μL, split ratio 50:1), the oven temperature was maintained at 150 °C for 1 min, then it was increased to 240 °C at 4 °C/min and kept at this temperature for 12 min. The standard FA methyl esters (37-component FAME Mix and PUFA number 2 from Sigma, Saint Louis, MO, USA) were run under the same conditions, and the subsequent retention times were used to identify the FA. FA was expressed as a percentage of the total FA identified.

### Experimental protocol

The experimental protocol was performed, as shown in Fig. 1. Adult flies (both genders) aged 1–4 days were used, and these were submitted to treatment, which had a duration of 7 days. The time of exposure to the diets and the addition of fat concentration to the diet was determined according to work performed with fat previously by our research group (Trindade de Paula et al., 2016). Therefore, the flies were divided into seven groups: (1) Regular diet (RD) (containing: corn flour, sugar, wheat germ, salt, powdered milk, and agar) (2) SHVF (HVF in the same proportion replaced values of fat of the RD), (3) HVF 10% (RD with 10% of HVF) (4)

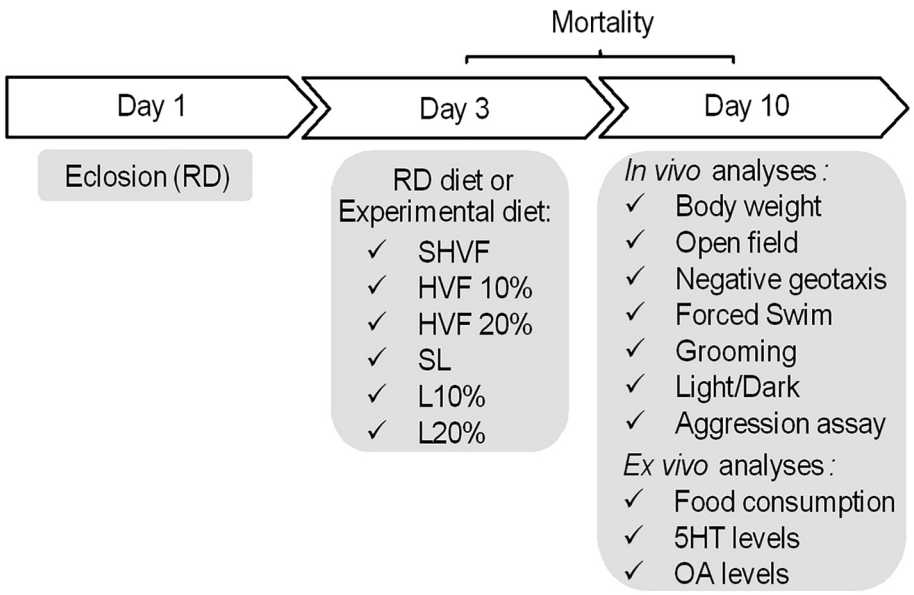


**Fig. 1.** Experimental design of the study.

HVF 20% (RD with 20% of HVF), (5) SL (values of fat of the RD were replaced by L in the same proportion), (6) L 10% (RD with 10% of L) and (7) L 20% (RD with 20% of L). The percentage values of fat were added to the RD, according to Table 1. The Fatty acid composition of HVF and L are detailed in table supplementary S1.

The values of macronutrients contained in the diets were calculated through the ADSNutri program using values referring to the Brazilian food composition table values given in Table 1.

### *In vivo* assays

Mortality and body weight**.** The flies' mortality rate was monitored daily by counting the number of dead flies every 24 h until the end of the treatment. The flies were transferred to fresh food every 2 days. Also, the body weight was analyzed at the beginning and end of the seven days of treatment. Approximately 150 flies per group were used for these analyses, totaling the sum of three independent experiments.

**Table 1.** Composition of the HVF diet and L diet

| | RD | SHFV | HFV 10% | HFV 20% | SL | L 10% | L 20% |
|---|---|---|---|---|---|---|---|
| Total (kcal/g) | **3.67** | **3.64** | **4.16** | **4.56** | **3.64** | **4.16** | **4.56** |
| Carbohydrates (%) | **79.44** | **80.34** | **63.81** | **12.87** | **80.34** | **63.81** | **12.87** |
| Fibers (%) | 6.44 | 6.31 | 6.44 | 6.44 | 6.31 | 6.44 | 6.44 |
| Proteins (%) | **11.4** | **10.79** | **9.21** | **7.70** | **10.79** | **9.21** | **7.70** |
| Total Fat (%) | **9.1** | **8.86** | **26.97** | **38.97** | **8.86** | **26.97** | **38.97** |
| *C10:0* | 0.12 | 0.02 | 0.16 | 0.18 | 0.02 | 0.15 | 0.16 |
| *C12:0* | 0.18 | 0.02 | 0.22 | 0.25 | 0.01 | 0.19 | 0.20 |
| *C14:0* | 0.63 | 0.02 | 0.63 | 0.63 | 0.11 | 0.85 | 1.00 |
| *C16:0* | 2.70 | 1.53 | 5.65 | 7.63 | 1.99 | 6.72 | 9.42 |
| *C16:1n7* | 0.09 | 0.02 | 0.09 | 0.09 | 0.15 | 0.41 | 0.63 |
| *C17:0* | 0.04 | 0.06 | 0.06 | 0.07 | 0.07 | 0.09 | 0.13 |
| *C17:1n5* | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.04 | 0.07 |
| *C18:0* | 0.81 | 1.29 | 3.66 | 5.57 | 0.95 | 2.84 | 4.21 |
| *C18:1n9t* | 0.16 | 1.12 | 2.81 | 4.59 | 0.03 | 0.24 | 0.28 |
| *C18:1n9c* | 2.05 | 3.52 | 9.69 | 14.82 | 3.33 | 9.24 | 14.08 |
| *C18:1n7* | 0.08 | 0.29 | 0.71 | 1.13 | 0.21 | 0.53 | 0.84 |
| *C18:2n6t* | 0.00 | 0.08 | 0.19 | 0.32 | 0.00 | 0.00 | 0.00 |
| *C18:2n6c* | 2.09 | 0.88 | 2.98 | 3.57 | 1.79 | 5.13 | 7.18 |
| *C18:3n3* | 0.15 | 0.03 | 0.15 | 0.15 | 0.09 | 0.28 | 0.38 |
| *C20:1n9* | 0.02 | 0.00 | 0.02 | 0.02 | 0.06 | 0.17 | 0.26 |
| *C20:3n6* | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.03 | 0.05 |
| *C20:4n6* | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.05 | 0.09 |

*Abbreviations:* RD, regular diet; SHVF, substitute hydrogenated vegetable fat; HVF, hydrogenated vegetable fat; SL, substitute lard; L, lard.

### Negative geotaxis

The climbing ability, used to analyze if locomotor damage in the flies, was evaluated according to the negative geotaxis test described by Paula et al. (2014). The flies were transferred separately to test tubes (length of 10 cm, a diameter of 1.5 cm), and then they were softly beaten to the bottom of the tube, and the time each fly took to climb up to 8 cm of the tube was measured. Fifteen flies per group were used, and each fly was tested five times in a 1-min interval. The data were analyzed according to the mean value between the times. Three independent experiments per group were performed.

### Open field test

The open field behavioral test was realized according to the method already described by Hirth (2010) with some adaptations. Each fly was positioned in an arena of 9 cm of diameter, divided into squares (1 cm $\times$ 1 cm) covered by a Petri dish. The exploratory activity was evaluated through the movement of the flies. The distance traveled during the time of 60 s was calculated by analyzing the number of squares that the fly had crossed. About 20 flies were used for each group, totaling four independent experiments.

### Forced swim test

The behavioral test was realized according to the method described by Neckameyer and Nieto-Romero (2015). A platform measuring 45 $\times$ 12 $mm^2$, completed with 4 mL of 0.08% SDS was used, where the flies were placed individually, and afterward, the following factors were observed during 5 min: First immobility time, total immobility time, total swimming time and the number of swim attempts. All these times were timed manually; in this test, 40 flies were evaluated individually per experimental group, totaling four independent experiments. The flies that remained immobile for a longer time to the situation were considered to present a depression-like behavior. For the validation of the test, after the evaluation time, the flies were removed from the swim platform and placed to a paper napkin, where the fly's recovery capacity was observed. The flies that we're incapable of walking normally were excluded from the final analysis.

Grooming. The analysis of grooming was realized, according to Tauber et al. (2011), with adaptations. About six flies were placed in an arena individually and allowed to acclimate for 1 min, where each fly was tested twice in a 1-min interval, it was evaluated how much time the flies spent realizing the cleaning act, during a total time of 2 min. The cleaning activity is a normal behavior performed by animals, but when performed excessively, it can serve as an indicator of stress and anxiety in wild and laboratory animals, being observed mainly with a mere to anxiety response (Estanislau, 2012; Smolinsky et al., 2009; Sousa et al., 2004). The data were analyzed according to the mean value between the times, a total of 24 flies per group were used, totalizing four independent experiments.

### Light/dark exploration test

This test was evaluated according to the method described by Neckameyer and Nieto-Romero (2015), with adaptations. A box of 6 $\times$ 1 cm, divided into a light compartment (16 W) and a dark compartment was used, the light and dark parts of the box were divided where there was a gateway so that the fly could choose in which side of the box it would prefer to stay. Six flies per group were placed individually in the light compartment of the box. After a period of adaptation, the amount of time spent in the dark compartment was registered. The fly was considered to be in the dark or light sections of the box when the head and at least half of the body were located within those sections. The flies *D.* are phototaxic; that is, they are more active during the daytime when there is clarity, so they are expected to remain in the light side of the box. Thus a larger exposure to the dark side of the box is considered an anxiety-like behavior (Araujo et al., 2018). A total of 24 flies per group were evaluated, totalizing four independent experiments.

### Aggression assay

The aggressiveness was evaluated according to the method of Edwards and Mackay (2006) adapted by Araujo et al. (2018). For this test, only male flies were used to avoid variability in the experiment, in addition to the fact that male flies are naturally more aggressive than female flies, bringing more effective results (Chen et al., 2002; Edwards et al., 2006). Pairs off male flies were placed in a circular arena with a radius of 45 mm and a height of 12 mm, and in the center of the arena there was a paper soaked with a drop of sucrose 5%. After an adaptation period in the arena, the aggressive events were observed during 5 min considering the following factors: leg extension from one fly to another resulting in physical contact, chasing, fast loading approach that leads to direct orientation, wing raising in response to proximity/approach of another fly, and high box impact interaction involving the front legs of both flies. Ten pairs of male flies were used per group for this evaluation, totalizing 30 doubles of males per group in a total of three independent experiments. The score corresponded to the number of aggressive encounters among the experimental flies.

### *Ex vivo* assays

Food consumption. The food intake was analyzed according to Sun et al. (2013) with adaptations. Flies of 1–7 days were used for the analysis. About five flies per group were placed in a diet containing 0.5% of blue dye (FD & C Blue Dye no. 1) added to their respective treatment group. The flies were allowed to feed for 30 min, and after that, they were transferred to Eppendorf tubes and anesthetized. The bodies were homogenized in 200 μL of distilled water, and then, centrifugation was performed at 12,000$\times g$ per 2 min. The absorbance of the supernatant was then measured at 625 nm using a spectrophotometer. A total of 20 flies were used per group, totalizing four independent experiments.

### 5HT and OA levels

The 5-HT and OA levels were determined by high-performance liquid chromatography with a Diode Array Detector (HPLC-DAD) model: Young Lin (YL 9100) equipped with a quaternary pump and autosampler. Thirty heads of flies were used per Eppendorf, and 288 μL of 0.9% NaCl and 12 μL of 0.5 M HCl were added. These heads were then homogenized with this solution for 1 minute on ice. The solution was centrifuged for 10 min at 7840×*g* at 4 °C. The supernatant was filtered with a 0.22 μm PTFE filter to a new eppendorf. Afterward, 200 μL were placed into an insert in a vial tube for analysis by HPLC-DAD (Bianchini et al., 2019).

### Statistical analysis

Data were analyzed according to the following model: $Y_{ij} = \mu + F_i + e_{ij}$ where: $Y_{ij}$ is the observed value; μ is the overall mean; $F_i$ is the fixed effect of addiction fat type; $e_{ij}$ is the random experimental error. Data normality was assessed using the Shapiro–Wilk test, and the homogeneity of the data was analyzed using the Bartlett's test. Data with normal and homogeneous distribution were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. The data with non-normal or heterogeneous distribution were evaluated by Kruskal–Wallis, followed by Dunn's post hoc test. Results were represented as box-and-whiskers plots, where the boxes represent the values from the 25th to 75th percentile, and the middle lines represent the median values. The mortality percent was determined using the Mantel–Cox log-rank test. Correlation analyzes were performed using Pearson's correlation coefficient. Differences between groups were considered significant when $p < 0.05$. GraphPad Prism software version 7 (San Diego, CA, USA) was used to perform these analyses.

## RESULTS

### Effect of diets with different concentrations of HVF and L on the mortality rate

The mortality analysis revealed that flies exposed to SHVF, HVF 10%, HVF 20%, SL, L 10%, and L 20% had a significant increase in the mortality percentage ($p < 0.05$) when compared with the RD group. In addition, the HVF 20% group had an increase in mortality when compared to its SHVF control, and the HVF 10% group. There was an increase in the mortality of flies exposed to HVF 10% compared to flies exposed to the same L (L 10%) concentration. Still, mortality was increased in the L 10% and L 20% groups compared to their SL control (Fig. 2).

Effect of diets with different concentrations of HVF and L on food consumption and body weight.

There was no significant difference in food intake between the flies exposed to different experimental diets (One-way ANOVA, $F_{6,\ 21} = 1.476$, $p = 0.23$; Table 2). Likewise, there was no significant difference in the body weight of flies (Kruskal–Wallis, $p = 0.09$; Table 2).

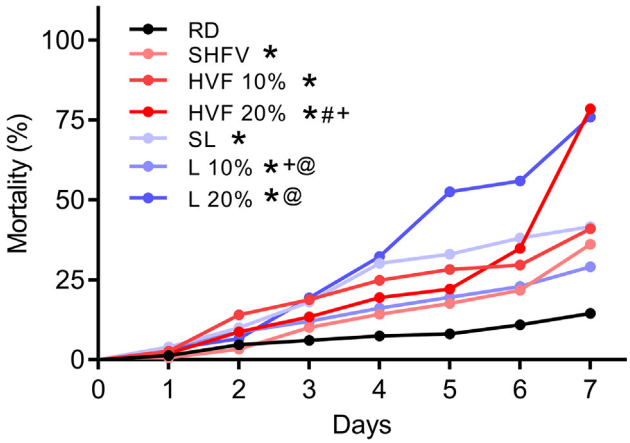


**Fig. 2.** Effect of different diets, Regular diet (RD), Hydrogenated vegetable fat (HVF), in concentrations of SHVF, HVF 10% and HVF 20%, besides Lard (L) in concentrations of SL, L 10% and L 20%, in the mortality percent of *Drosophila melanogaster* flies. A total of 150 flies per group were used, totaling the sum of three independent experiments. The measure of life time was determined comparing the curves of mortality of Mantel–Cox log-rank tests. $^{*}p < 0.05$, indicates significant difference in relation to the RD group; $^{\#}p < 0.05$, indicates significant difference in relation to the SHVF group; $^{+}p < 0.05$, indicates significant difference in relation to the HVF 10% group and $^{@}p < 0.05$, indicates significant difference in relation to the SL group.

**Table 2.** Food consumption and body weight of flies

| | Food consumption | Body Weight |
|---|---|---|
| RD | 115.7 ± 18.44 | 100.6 ± 2.60 |
| SHVF | 105.1 ± 27.69 | 92.71 ± 27.27 |
| HVF 10% | 75.32 ± 12.21 | 63.26 ± 19.02 |
| HVF 20% | 74.04 ± 12.30 | 143.0 ± 62.02 |
| SL | 98.65 ± 11.61 | 159.2 ± 22.78 |
| L 10% | 113.7 ± 18.29 | 101.2 ± 31.00 |
| L 20% | 59.71 ± 20.18 | −12.11 ± 55.77 |

*Abbreviations:* RD, regular diet; SHVF, substitute hydrogenated vegetable fat; HVF, hydrogenated vegetable fat; SL, substitute lard; L, lard. To evaluate the food consumption a total of 20 flies per group were used, totaling the sum of four independent experiments. To evaluate the body weight a total of 150 flies per group were used, totaling the sum of three independent experiments. Values are expressed by (%) of the RD, and ± standard error of means. For analysis the food consumption the significance was determined by a one-way analysis of variance (ANOVA), followed by a Tukey test. For determination of the body weight, the significance was determined by nonparametric analysis Kruskal–Wallis.

Effect of diets with different concentrations of HVF and L on the levels of 5HT and OA.

There was a significant reduction of the 5HT levels in the flies exposed to HVF at all concentrations and in the flies exposed to L at the concentration of 20% when compared with the RD group (One-way ANOVA, $F_{6,\ 23} = 5.49$, $p = 0.001$; Fig. 3A). Regarding OA, our results showed a reduction in OA levels in both fats, in all concentrations, when compared to the RD group. (One-way ANOVA, $F_{6,\ 21} = 7.57$, $p = 0.0002$; Fig. 3B)

### Effect of diets with different concentrations of HVF and L on depression-like behavior

It was observed a decrease in the latency of the first immobility and in the total swimming in the flies exposed

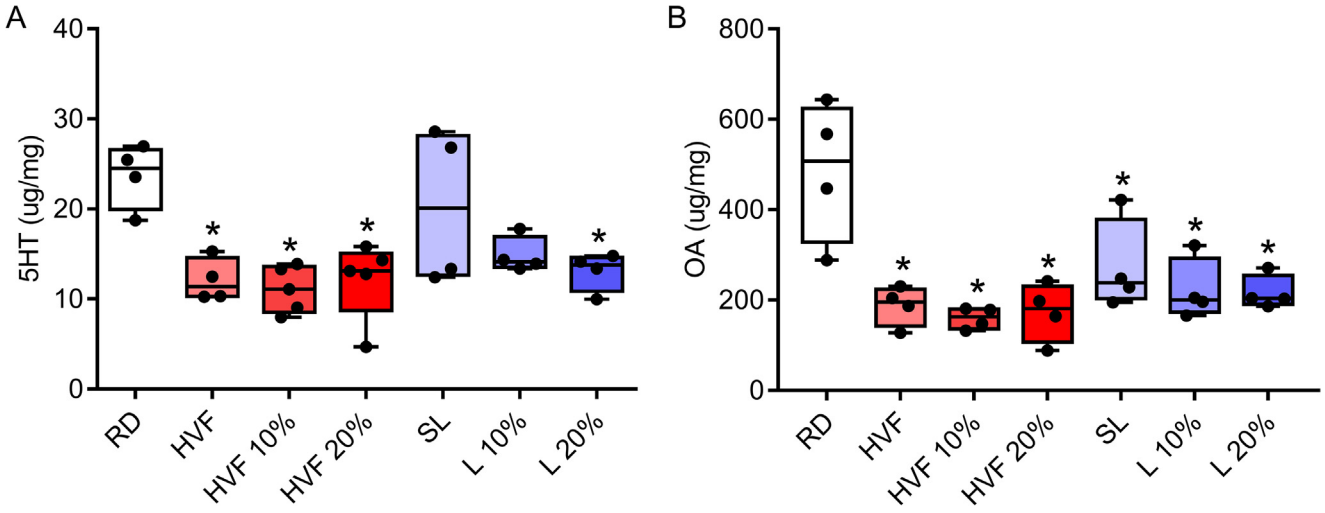


**Fig. 3.** Effect of the different diets, Regular diet (RD), Hydrogenated vegetable fat (HVF), in concentrations of SHVF, HVF 10% and HVF 20%, besides Lard (L) in concentrations of SL, L 10% and L 20%, in 5HT and OA levels in the head of the *Drosophila melanogaster* flies. **(A)** 5HT levels. **(B)** OA levels. To evaluate the 5HT and OA levels, a total of 120 flies per group were used, totaling the sum of four independent experiments. The boxes represent the values from the 25th to 75th percentile. The middle lines represent the median values. The significance was determined by a one-way analysis of variance (ANOVA), followed by Tukey test. *$p < 0.05$, indicates a significant difference in relation to RD group.

to both HVF and L rich diets at all concentrations when compared with the RD group. In addition, there was a decrease in the latency of the first immobility and the total swimming in the HVF 20% and L group at concentrations of 10% and 20% compared to their control, SHVF and SL respectively (One-way ANOVA, $F_{6,\ 21} = 140.9$, $p < 0.0001$; Fig. 4A; $F_{6,\ 21} = 127.8$, $p < 0.0001$; Fig. 4B). On the other hand, there was no significant difference in the number of bouts between the experimental groups (Kruskal–Wallis, $p = 0.63$; Fig. 4C). However, there was an increase in the total immobility time in flies exposed to diets rich in HVF and L in all concentrations compared to the RD group. Also, there was an increase in immobility time in the HVF 20% groups when compared to their control, SHVF. Similarly, this increase in immobility time occurred in groups exposed to L at concentrations of 10% and 20% when compared to their control, SL (One-way ANOVA, $F_{6,\ 21} = 127.8$, $p < 0.0001$; Fig. 4D).

### Effect of diets with different concentrations of HVF and L on anxiety-like behavior

There was a significant increase in grooming in the concentrations of HVF 10% and HFV 20% when compared to the RD group. Also, there was an increase in grooming activity in the HVF groups at concentrations of 10% and 20%, when compared to their control, SHVF. The groups exposed to HVF 10% and HVF 20% showed higher grooming activity when compared to the groups L 10% and L 20%, respectively (One-way ANOVA, $F_{6,\ 21} = 15.23$, $p < 0.0001$; Fig. 5A). In the light/dark test, there was a significant increase in the dark time in flies exposed to 20% HVF when compared to the RD group (Kruskal–Wallis, $p = 0.001$; Fig. 5B).

### Effect of diets with different concentrations of HVF and L on climbing ability and exploratory behavior

The climbing capacity of flies exposed to different experimental diets was not affected, as analyzed by the negative geotaxis test (Kruskal–Wallis, $p = 0.04$; Fig. 6A). Likewise, there were no changes in locomotor activity between flies, as analyzed by the open field test (Kruskal–Wallis, $p = 0.07$; Fig. 6B).

### Effect of diets with different concentrations of HVF and L on aggressive behavior

Our results show that aggressive behavior was increased in flies exposed to a diet containing HVF 20% compared to the RD group; there is no significant difference in the other groups (Kruskal–Wallis, $p = 0.007$; Fig. 7).

### Effect of diets with different concentrations of HVF and L on Pearson's correlation analysis ($r$) between the biogenic amines 5HT and OA and behavioral paradigms

The Pearson's correlation analysis (Table 3) showed a significant negative correlation between 5HT and aggressive behavior ($r = -0.6867$; $p = 0.0006$), 5HT and immobility time ($r = -0.6638$; $p = 0.0001$), 5HT and negative geotaxis ($r = -0.6918$; $p = 0.0005$), 5HT and light/dark ($r = -0.6380$; $p = 0.0003$), 5HT and grooming ($r = -0.4635$; $p = 0.013$), OA and aggressive behavior ($r = -0.7672$; $p < 0.0001$), OA and immobility time ($r = -0.8532$; $p = < 0.0001$), OA and negative geotaxis ($r = -0.6999$; $p = 0.0004$), OA and light/dark ($r = -0.7171$; $p = < 0.0001$), and OA and grooming ($r = -0.6270$; $p = 0.0004$). In addition, the Pearson's correlation analysis showed a significant positive correlation between 5HT and open field ($r = 0.4607$; $p = 0.01$) and OA and open field ($r = 0.4797$; $p = 0.009$).

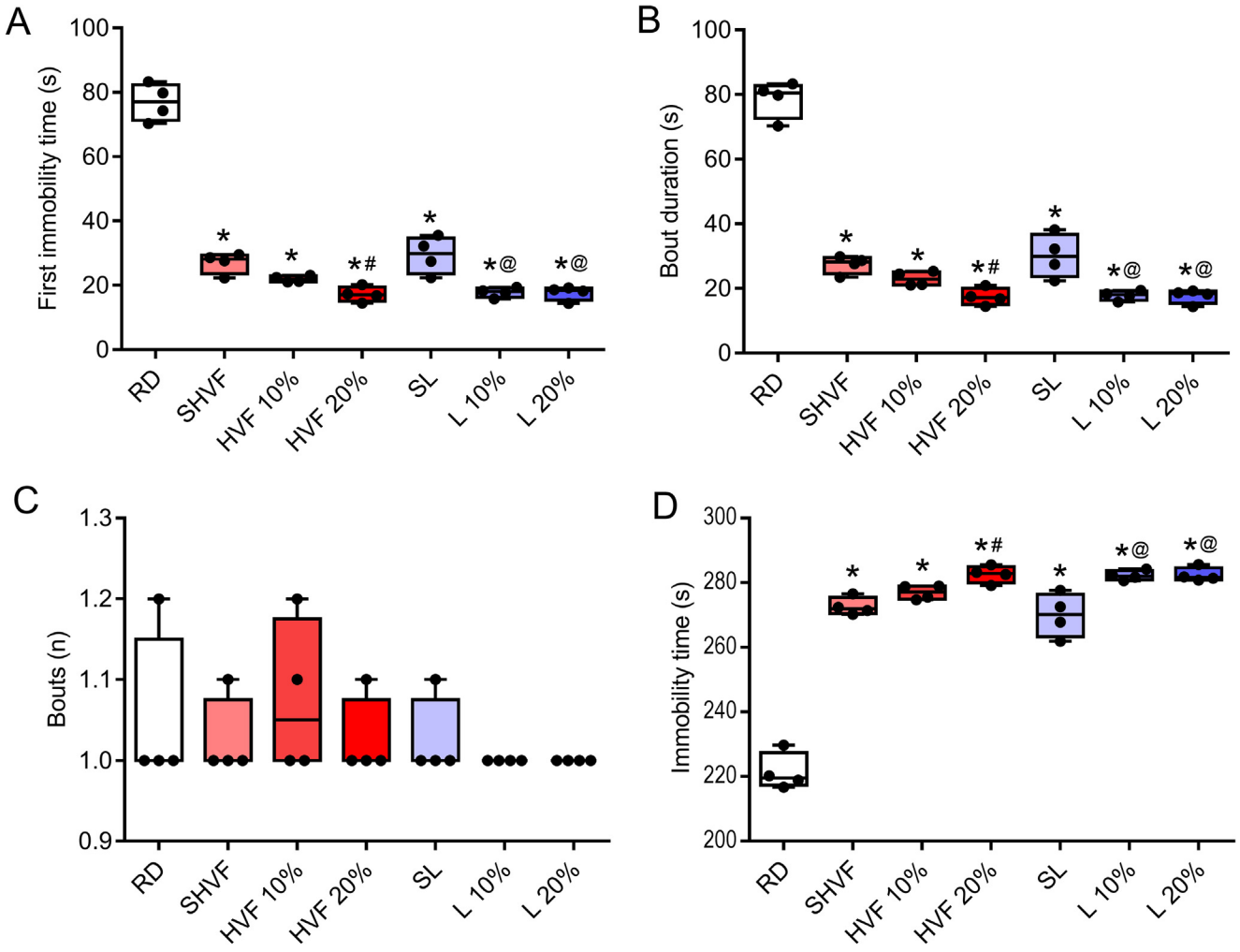


**Fig. 4.** Effect of different diets, Regular diet (RD), Hydrogenated vegetable fat (HVF), in concentrations of SHVF, HVF 10% and HVF 20%, besides Lard (L) in concentrations of SL, L 10% and L 20%, in the forced swim test in *Drosophila melanogaster* flies. **(A)** First immobility time, **(B)** Bout duration, **(C)** Number of bout, **(D)** Immobility time. A total of 40 flies per group were used, totaling the sum of four independent experiments. The boxes represent the values from the 25th to 75th percentile. The middle lines represent the median values. The significance was determined by one-way analysis of variance (ANOVA), followed by Tukey test for first immobility time, bout duration and immobility time. For number of bout the significance was determined by nonparametric analysis Kruskal–Wallis. $^{*}p < 0.05$, indicates significant difference in relation to the RD group; $^{\#}p < 0.05$, indicates significant difference in relation to the SHVF group; $^{@}p < 0.05$, indicates significant difference in relation to the SL group.

## DISCUSSION

In this study, we evaluated the effects of diet consumption with different percentages of two types of fat, usually consumed in a great amount by the population. Our intention was to determine the influence of these different diets on the emergence of behavioral changes related to neuropsychological disorders in the *Drosophila* model. Our findings showed that a diet with high-fat content could contribute to the development of behavior disturbances, which were observed in the parameters evaluated, such as, increase in depressive and anxious behavior and an increase in the number of aggressive events, however, with no locomotor impairment. A significant negative correlation was observed between 5HT or OA levels and aggressiveness, negative geotaxis, immobility time, and light/dark or grooming behaviors. Also, there was a significant positive correlation between 5HT and OA levels in the flies' exploratory activity. Curiously, the chronic consumption of the different diets wasn't related to the increase in body weight; however, there was a decrease in the flies' life expectancy.

It was observed that diets with different fat concentrations decreased the flies useful life, which was evaluated by the daily mortality percentage. Besides, this reduction in helpful life was increased as the concentration of fats increased in experimental diets. This reduction may be due to the range of metabolic changes caused by the high consumption of fat. We can observe that the decrease in life expectancy was observed in both types of fat and at all the evaluated concentrations. This result may also be related to the type of lipid consumed in the diet offered to the flies, since there already are data related to the reduction of life expectancy during the increase in the consumption

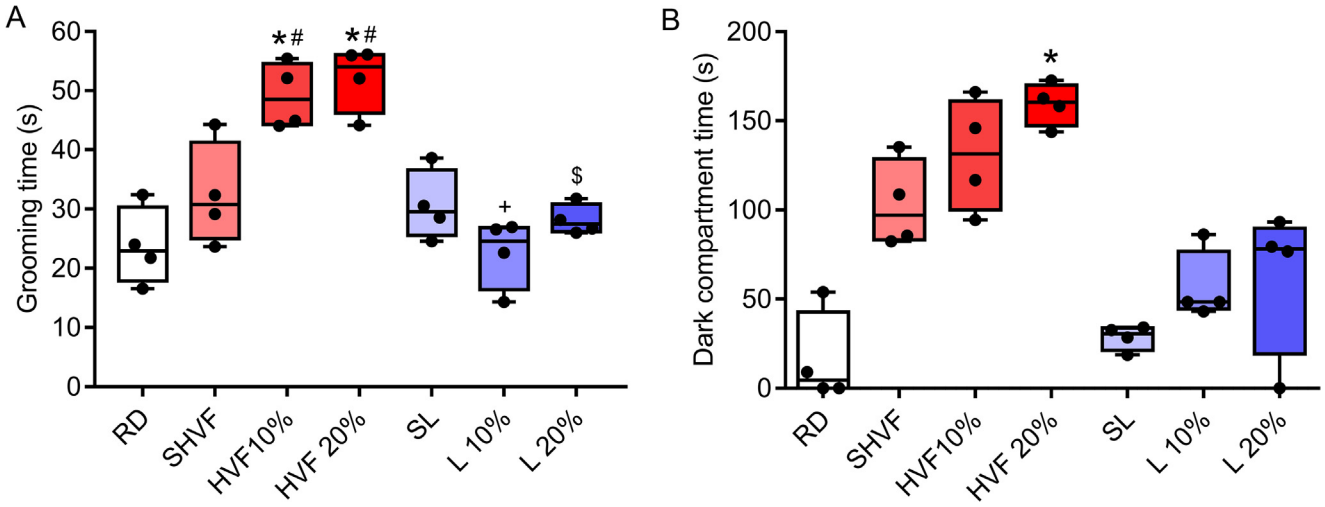


**Fig. 5.** Effect of different diets, Regular diet (RD), Hydrogenated vegetable fat (HVF), in concentrations of SHVF, HVF 10% and HVF 20%, besides Lard (L) in concentrations of SL, L 10% and L 20%, in the anxiety-like behavior in *Drosophila melanogaster* flies. **(A)** Grooming test, **(B)** Light and dark test. A total of 24 flies per group were used for each test, totaling the sum of four independent experiments. The boxes represent the values from the 25th to 75th percentile. The middle lines represent the median values. The significance was determined by a one-way analysis of variance (ANOVA), followed by a Tukey test, for grooming test. For light and dark test, the significance was determined by nonparametric analysis Kruskal–Wallis. $^{*}p < 0.05$, indicates significant difference in relation to the RD group; $^{\#}p < 0.05$, indicates significant difference in relation to the SHVF group; $^{+}p < 0.05$, indicates significant difference in relation to the HVF 10% group and $^{\$}p < 0.05$, indicates significant difference in relation to the HVF 20% group.

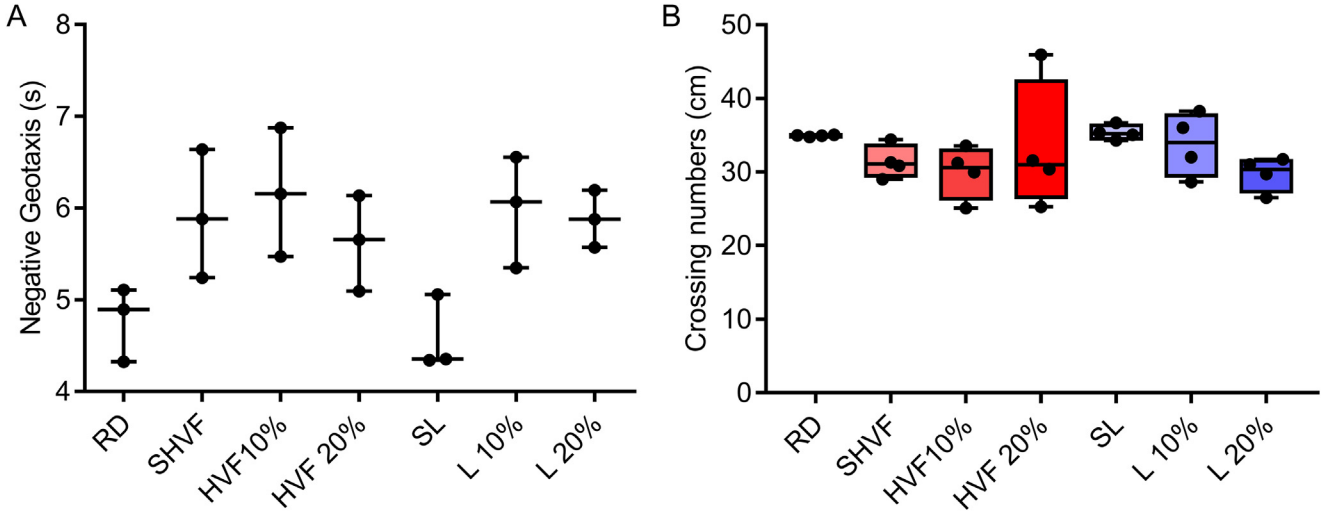


**Fig. 6.** Effect of the different diets, Regular diet (RD), Hydrogenated vegetable fat (HVF), in concentrations of SHVF, HVF 10% and HVF 20%, besides Lard (L) in concentrations of SL, L 10% and L 20%, in the locomotive ability test in *Drosophila melanogaster* flies. **(A)** Negative geotaxis test, **(B)** Open field test. To evaluate the ability to scale a total of 15 flies per group were used, totaling the sum of three independent experiments. To evaluate the locomotor, damage a total of 20 flies per group were used, totaling the sum of four independent experiments. The boxes represent the values from the 25th to 75th percentile. The middle lines represent the median values. The significance was determined by nonparametric analysis Kruskal–Wallis. It was considered significant when $p < 0.05$.

of saturated FA (Trindade de Paula et al., 2016), and negative consequences caused by high consumption of trans FA (Semma, 2002).

Thus, we can observe that the amount of fat offered in the diet is of importance and the fat's FAs composition since different lipids play important roles in the brain metabolism and structure (Labouesse et al., 2013). Therefore, in our study, we evaluated the FA composition in the diet offered to the flies. We observed a deficiency of essential FA that is necessary for brain health, such as α-linolenic acid, also known as omega 3. This deficiency is related to increased incorporation of trans isomers into brain structures, which leads to neurotransmitter signaling disorders (Grandgirard et al., 1994). In our study, the trans FA was found in high amounts in HVF-enriched diets, since the recommended amount of trans fat does not exceed 1% of the intake (Liu et al., 2017). Also, the deficiency of essential FAs may be related to the reduction of 5-HT and OA monoamines observed here, since prolonged omega-3 FA deprivation and substitution

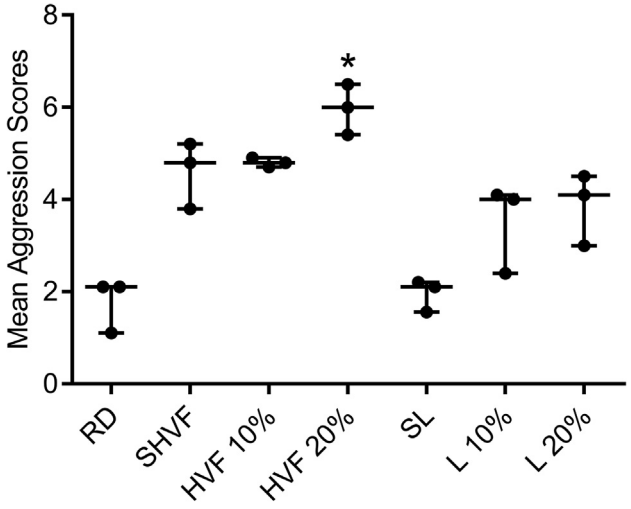


**Fig. 7.** Effect of different diets, Regular diet (RD), Hydrogenated vegetable fat (HVF), in concentrations of SHVF, HVF 10% and HVF 20%, besides Lard (L) in concentrations of SL, L 10% and L 20%, in aggressive behavior of *Drosophila melanogaster* flies. To evaluate the aggressive behavior, a total of 30 pairs of male flies per group were used, totaling the sum of three independent experiments. The boxes represent the values from the 25th to 75th percentile. The middle lines represent the median values. The significance was determined by nonparametric analysis Kruskal–Wallis. $*p < 0.05$, indicates significant difference in relation to the RD group.

**Table 3.** Effects of HVF and L on Pearson's correlation analysis (*r*) between the biogenic amines 5HT and OA and behavioral paradigms

| Biogenic amines and behavioral parameters | *r* | *P* | *n* |
|---|---|---|---|
| 5HT × Immobility Time | −0.6638 | < 0.0001* | 28 |
| 5HT × Light/Dark | −0.6380 | 0.0003* | 28 |
| 5HT × Grooming | −0.4635 | 0.0130* | 28 |
| 5HT × Negative Geotaxis | −0.6918 | 0.0005* | 21 |
| 5HT × Open Field | 0.4607 | 0.0179* | 26 |
| 5HT × Agressive Behavior | −0.6867 | 0.0006* | 21 |
| OA × Immobility Time | −0.8532 | < 0.0001* | 28 |
| OA × Light/Dark | −0.7171 | < 0.0001* | 28 |
| OA × Grooming | −0.6270 | 0.0004* | 28 |
| OA × Negative Geotaxis | −0.6999 | 0.0004* | 21 |
| OA × Open field | 0.4797 | 0.0098* | 28 |
| OA × Agressive Behavior | −0.7672 | < 0.0001* | 21 |

Serotonin (5-HT); Octopamin (OA); Pearson's correlation analysis (*r*). It is considered significant when $*p < 0.05$.

impairs the brain monoamine system. This system has an essential role in mood changes, being mainly associated with depressive disorders. These disease affects a large part of the world population and can lead to death (Chalon, 2006). It is also involved in disorders such as anxiety, aggression, and impulsivity (Kim, 2008). Because of this, we chose to evaluate the levels of 5-HT and OA biogenic amines in the head of the flies exposed to the experimental diets correlating with changes in their behavior. In our study, a significant reduction in 5-HT levels was observed in the heads of the flies receiving HVF diets at concentrations of SHVF, HVF 10% and HVF 20%, but in the flies receiving the L diet there was only a reduction in the group with higher concentration, L 20% when compared with the RD group. It is suggested that this occurs because the membrane is altered according to the FAs consumed in the diet and the trans FA obtained from processed food has a different conformation than a natural FA. Thus it modifies the membrane structure and permeability, consequently causing the alteration in neurotransmission as observed here (Ginter and Simko, 2016).

Similarly, groups that received different types of fat showed a significant reduction in OA levels. However, this reduction was observed at all concentrations when compared with the RD group. This result is of great relevance since the OA found in *Drosophila* is analogous to noradrenaline/norepinephrine, which is found in vertebrates and plays important roles, especially regarding depression and anxiety disorders (Busch et al., 2009; Huang et al., 2019; Lee et al., 2003; Shelton, 2018). However, in *Drosophila*, OA is recognized for playing roles related to memory, locomotion, reproduction, among others (Claßen and Scholz, 2018; Pauls et al., 2018), with scarcity in studies that relate this amine to depressive and anxiety states in this model, making it a given to be considered.

In our study, the flies exposed to the HFV and L diets at all concentrations had depression-like behaviors, observed through time immobility in the forced swimming test, also, it was observed that when the fat concentration was higher, this resulted in a longer immobile time of the flies on the platform. Such findings are according to a study carried out with rodents, where a diet that was rich in fat caused behaviors similar to depression, also evaluated through the forced swim test (Yang et al., 2013). Besides, there was a negative correlation between immobility time and the 5-HT assessed levels here. Related to this, 5-HT neurons project widely throughout the brain to innervate key regions involved in depression and anxiety, according to other studies, the vulnerability to these disorders is related to the low serotoninergic function (Carver et al., 2009; Zemdegs et al., 2016). Similarly, a negative correlation was observed between OA levels and immobility time in the flies exposed to a different type of fat. This is in line with other studies where the reduction of its homologous, norepinephrine in vertebrates, is associated with depressive situations where rats with stress-induced depressive symptoms had norepinephrine levels decreased (Huang et al., 2019). Thus, it is shown for the first time that a reduction in OA levels may be associated with type-depressive behavior in the *D. melanogaster* model.

Frequently, patients in a depressive state can present anxiety associated with the disorder (Dunner et al., 2003). Therefore, we evaluated a behavioral state similar to anxiety, and our results showed that flies exposed to the HVF diet in concentrations of 10% and 20% showed a higher grooming behavior, when compared to the RD group and the L groups, in the same concentrations, L 10%, and L 20%, respectively. On the other hand, the light/dark test showed that the flies exposed to the HVF 20% diet remained on the dark side of the box for a longer time when compared to the RD group. Related to that, a study realized with rats showed incorporation of trans FA in the

brain of animals (0,30%), causing the modification of the activity of Na + K + -ATPase and so, alterations in the parameters related to anxiety and memory of animals (Teixeira et al., 2011). These results are in accordance with studies realized in rodents exposed to high-fat diets at long-term, where they evaluate the effects related to psychological disorders, and it is observed that the rats exposed to these diets had behaviors like anxiety and anhedonia, without affecting the locomotor activity (Dutheil et al., 2016; Krishna et al., 2015).

In the same way, we evaluated the locomotor activity and climbing ability of the flies, performed in the open field and negative geotaxis tests, respectively. In our results, no locomotor declines were observed in the flies, which is considered a positive and fundamental result, because that way, the previous tests related to anxiety and depression become valid, since there was not any influence of locomotor damage in the flies (Araujo et al., 2018).

Regarding behavioral changes, we evaluated the flies' aggressive behavior, where a greater number of aggressive events were observed in the flies exposed to HVF in the concentration of 20%. In addition, a negative correlation was observed between the number of aggressive events and the 5HT and OA levels evaluated here. Aggressive behavior is considered of great importance in several factors such as food defense, companion, and progeny, among others. However, there is a considerable increase in the number of aggressive episodes in patients with behavioral disorders, associated with a reduction in 5HT levels (Nelson and Chiavegatto, 2001; Edwards et al., 2006). In this sense, the low serotoninergic function allows the brain areas related to emotion and mood to be more active, intensifying actions and emotions, which could explain the larger episode of combat events found here (Spoont, 1992). On the other hand, this result contrasts with other findings related to OA concentrations, where a reduction in OA levels would be associated with the decrease of aggressive events. However, the role of OA in aggression is still not well understood as such changes in aggressiveness depend on the context (Hoyer et al., 2008).

Furthermore, the effects of a high-fat diet on aggressive behavior can be mediated by innumerable factors, from bodyweight to even changes in cell membrane structure, since dietary FA can be incorporated into the lipid bilayer, altering their fluidity and cellular functionality, it can lead to possible changes in the release of neurotransmitters responsible for mood, such as the 5HT neurotransmitter evaluated here (Giriko et al., 2013; Sullivan et al., 2014).

In addition to the behavioral changes caused by the diet with different percentages of fat observed in this study, studies have shown that the high-fat diet is capable of causing other types of behavioral changes in flies, such as changes in behavior and sexual characteristics (Schultzhaus et al., 2018), reduced olfactory sensitivity (Jung et al., 2018) and decline in memory (Rivera et al., 2019), thus demonstrating that the fat offered in the diet is capable of leading to a range of neurobehavioral changes.

Our results show that the AF offered in the diet has great importance in brain health, and can be responsible for behavioral changes, being the trans type AF, considered the most harmful to health, as demonstrated in this work. Thus, it is concluded that D. melanogaster can serve as a useful model for the understanding of psychiatric disorders and that the type of FA offered in the diet can influence these disorders, demonstrating the importance of the composition of the FAs in the neural pathways, which can influence the signaling of neurotransmitters such as the 5HT and OA assessed here, and thus, cause behavioral changes.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in the present work.

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## AUTHOR CONTRIBUTIONS

MP access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

LBM and MP conceived and designed the project.

LBM, MRP, VCB, SMA, MMD, EAM, and SFC performed behavioral experiments.

MCG and RR performed the biochemical measurements and acquisition of data.

SS and TE performed the lipid content analysis.

LBM, GPG, and MP interpreted of data and statistical analysis.

LBM drafting of the manuscript.

Critical revision of the manuscript for important intellectual content: GPG and MP.

## REFERENCES

Adams J, White M (2015) Characterisation of UK diets according to degree of food processing and associations with socio-demographics and obesity: cross-sectional analysis of UK National Diet and Nutrition Survey (2008–12). Int J Behav Nutr Phys Act 12:160.

Costa AGV, Bressan J, Sabarense CM (2006) Archivos latinoamericanos de nutrición. Arch Latinoam Nutr 56:12–21.

Araujo SM, Poetini MR, Bortolotto VC, de Freitas CS, Pinheiro FC, Meichtry LB, de Almeida FP, Santos Musachio EA, de Paula MT, Prigol M (2018) Chronic unpredictable mild stress-induced depressive-like behavior and dysregulation of brain levels of biogenic amines in *Drosophila melanogaster*. Behav Brain Res 351:104–113.

Atella GC, Majerowicz D, Gondim KC (2012) CAPÍTULO 6 Metabolismo de Lipídeos. http://www.inctem.bioqmed.ufrj.br/

images/documentos/biblioteca/Capitulo_6_Metabolismo_de_Lipideos.pdf.
Benton R (2008) Chemical sensing in *Drosophila*. Curr Opin Neurobiol 18:357–363.
Bianchini MC, Gularte COA, Nogara PA, Krum BN, Gayer MC, Bridi JC, Roos DH, Roehrs R, Fachinetto R, Pinton S, Ávila DS, Hirth F, Rocha JBT, Puntel RL (2019) Thimerosal inhibits *Drosophila melanogaster* tyrosine hydroxylase (*Dm* TyrH) leading to changes in dopamine levels and impaired motor behavior: implications for neurotoxicity. Metallomics 11:362–374.
Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917.
Busch S, Selcho M, Ito K, Tanimoto H (2009) A map of octopaminergic neurons in the *Drosophila* brain. J Comp Neurol 513:643–667.
Campos AC, Fogaca MV, Aguiar DC, Guimaraes FS, Campos AC, Fogaca MV, Aguiar DC, Guimaraes FS (2013) Animal models of anxiety disorders and stress. Rev Bras Psiquiatr 35:S101–S111.
Carver CS, Johnson SL, Joormann J (2009) Two-mode models of self-regulation as a tool for conceptualizing effects of the serotonin system in normal behavior and diverse disorders. Curr Dir Psychol Sci 18:195–199.
Chalon S (2006) Omega-3 fatty acids and monoamine neurotransmission. Prostaglandins, Leukot Essent Fat Acids 75:259–269.
Chen S, Lee AY, Bowens NM, Huber R, Kravitz EA (2002) Fighting fruit flies: A model system for the study of aggression. Proc Natl Acad Sci U S A 99:5664–5668.
Claßen G, Scholz H (2018) Octopamine shifts the behavioral response from indecision to approach or aversion in *Drosophila melanogaster*. Front Behav Neurosci 12:131.
Dunner DL, Goldstein DJ, Mallinckrodt C, Lu Y, Detke MJ (2003) Duloxetine in treatment of anxiety symptoms associated with depression. Depress Anxiety 18:53–61.
Dutheil S, Ota KT, Wohleb ES, Rasmussen K, Duman RS (2016) High-fat diet induced anxiety and anhedonia: impact on brain homeostasis and inflammation. Neuropsychopharmacology 41:1874–1887.
Edwards AC, Rollmann SM, Morgan TJ, Mackay TFC (2006) Quantitative genomics of aggressive behavior in *Drosophila melanogaster*. PLoS Genet 2 e154.
Estanislau C (2012) Cues to the usefulness of grooming behavior in the evaluation of anxiety in the elevated plus-maze. Psychol Neurosci 5:105–112.
Ginter E, Simko V (2016) New data on harmful effects of trans-fatty acids. Bratisl Lek Listy 117:251–253.
Giriko CÁ, Andreoli CA, Mennitti LV, Hosoume LF, Souto T dos S, da Silva AV, Mendes-da-Silva C (2013) Delayed physical and neurobehavioral development and increased aggressive and depression-like behaviors in the rat offspring of dams fed a high-fat diet. Int J Dev Neurosci 31:731–739.
Grandgirard A, Bourre JM, Julliard F, Homayoun P, Dumont O, Piciotti M, Sebedio JL (1994) Incorporation of trans long-chain n-3 polyunsaturated fatty acids in rat brain structures and retina. Lipids 29:251–258.
Hartman L, Lago RC (1973) Rapid preparation of fatty acid methyl esters from lipids. Lab Pract 22:475–476.
Haygert P, Roversi K, Milanesi LH, Maurer LH, Camponogara C, Duarte T, Barcelos RCS, Emanuelli T, Oliveira SM, Duarte MMMF, Trevizol F, Burger ME (2018) Can the dietary fat type facilitate memory impairments in adulthood? A comparative study between Mediterranean and Western-based diet in rats. J Nutr Biochem 59:104–113.
Hirth F (2010) Drosophila melanogaster in the study of human neurodegeneration. CNS Neurol Disord Drug Targets 9:504–523.
Hoyer SC, Eckart A, Herrel A, Zars T, Fischer SA, Hardie SL, Heisenberg M (2008) Octopamine in male aggression of Drosophila. Curr Biol 18:159–167.
Huang D, Zhang L, Yang J, Luo Y, Cui T, Du T, Jiang X (2019) Evaluation on monoamine neurotransmitters changes in depression rats given with sertraline, meloxicam or/and caffeic acid. Genes Dis 6:167–175.
Iqbal MP (2014) Trans fatty acids – a risk factor for cardiovascular disease. Pak J Med Sci 30:194–197.
Jung J, Kim D-I, Han G-Y, Kwon H (2018) The effects of high fat diet-induced stress on olfactory sensitivity, behaviors, and transcriptional profiling in Drosophila melanogaster. Int J Mol Sci 19:2855.
Kim JJ (2008) Serotonin, stress, and conditioning. Biol Psychiatry 63:819–820.
Krishna S, Keralapurath MM, Lin Z, Wagner JJ, de La Serre CB, Harn DA, Filipov NM (2015) Neurochemical and electrophysiological deficits in the ventral hippocampus and selective behavioral alterations caused by high-fat diet in female C57BL/6 mice. Neuroscience 297:170–181.
Labouesse MA, Stadlbauer U, Langhans W, Meyer U (2013) Chronic high fat diet consumption impairs sensorimotor gating in mice. Psychoneuroendocrinology 38:2562–2574.
Lam MCL, Adams J (2017) Association between home food preparation skills and behaviour, and consumption of ultra-processed foods: Cross-sectional analysis of the UK National Diet and nutrition survey (2008–2009). Int J Behav Nutr Phys Act 14:68.
Lee H-G, Seong C-S, Kim Y-C, Davis RL, Han K-A (2003) Octopamine receptor OAMB is required for ovulation in Drosophila melanogaster. Dev Biol 264:179–190.
List GR, Marangoni AG (2017) Trans fat replacements in foods. Available at: http://lipidlibrary.aocs.org/Food/content.cfm?ItemNumber=41540 [Accessed December 11, 2018].
Liu AG, Ford NA, Hu FB, Zelman KM, Mozaffarian D, Kris-Etherton PM (2017) A healthy approach to dietary fats: understanding the science and taking action to reduce consumer confusion. Nutr J 16:53.
Liu Z, Huang X (2013) Lipid metabolism in Drosophila: development and disease. Acta Biochim Biophys Sin (Shanghai) 45:44–50.
Morales MM (2008) Métodos alternativos à utilização de animais em pesquisa científica: mito ou realidade? Cienc Cult 60:33–36.
Neckameyer WS, Nieto-Romero R (2015) Response to stress in *Drosophila* is mediated by gender, age and stress paradigm. Stress 18:254–266.
Nelson RJ, Chiavegatto S (2001) Molecular basis of aggression. Trends Neurosci 24:713–719.
Pandey UB, Nichols CD (2011) Human disease models in drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol Rev 63:411–436.
Paula MT, Zemolin AP, Vargas AP, Golombieski RM, Loreto ELS, Saidelles AP, Picoloto RS, Flores EMM, Pereira AB, Rocha JBT, Merritt TJS, Franco JL, Posser T (2014) Effects of Hg(II) exposure on MAPK phosphorylation and antioxidant system in *D. melanogaster*. Environ Toxicol 29:621–630.
Pauls D, Blechschmidt C, Frantzmann F, el Jundi B, Selcho M (2018) A comprehensive anatomical map of the peripheral octopaminergic/tyraminergic system of Drosophila melanogaster. Sci Rep 8:15314.
Rivera O, McHan L, Konadu B, Patel S, Sint Jago S, Talbert ME (2019) A high-fat diet impacts memory and gene expression of the head in mated female Drosophila melanogaster. J Comp Physiol B Biochem Syst Environ Physiol 189:179–198.
Schultzhaus JN, Bennett CJ, Iftikhar H, Yew JY, Mallett J, Carney GE (2018) High fat diet alters Drosophila melanogaster sexual behavior and traits: Decreased attractiveness and changes in pheromone profiles. Sci Rep 8:5387.
Semma M (2002) Trans fatty acids: properties, benefits and risks. J Health Sci 48(1):7–13.
Sharma S, Zhuang Y, Gomez-Pinilla F (2012) High-fat diet transition reduces brain DHA levels associated with altered brain plasticity and behaviour. Sci Rep 2:431.
Shelton RC (2018) Serotonin and norepinephrine reuptake inhibitors. In: Handbook of experimental pharmacology, pp 145–180.
Siri-Tarino PW, Sun Q, Hu FB, Krauss RM (2010) Saturated fat, carbohydrate, and cardiovascular disease. Am J Clin Nutr 91:502–509.

Smolinsky AN, Bergner CL, LaPorte JL, Kalueff AV (2009) Analysis of grooming behavior and its utility in studying animal stress. Anxiety Depress:21–36.

Sousa FCF, Melo CTV, Monteiro AP, Lima VTM, Gutierrez SJC, Pereira BA, Barbosa-Filho JM, Vasconcelos SMM, Fonteles MF, Viana GSB (2004) Antianxiety and antidepressant effects of riparin III from Aniba riparia (Nees) Mez (Lauraceae) in mice. Pharmacol Biochem Behav 78:27–33.

Spoont MR (1992) Modulatory role of serotonin in neural information processing: implications for human psychopathology. Psychol Bull 112:330–350.

Sullivan EL, Nousen EK, Chamlou KA (2014) Maternal high fat diet consumption during the perinatal period programs offspring behavior. Physiol Behav 123:236–242.

Sun Y, Yolitz J, Wang C, Spangler E, Zhan M, Zou S (2013) Aging studies in Drosophila Melanogaster. In: Methods in molecular biology (Clifton, N.J.). p. 77–93.

Tauber JM, Vanlandingham PA, Zhang B (2011) Elevated levels of the vesicular monoamine transporter and a novel repetitive behavior in the Drosophila model of fragile X syndrome Zars T, ed. PLoS One 6 e27100.

Teixeira AM, Pase CS, Boufleur N, Roversi K, Barcelos RCS, Benvegnú DM, Segat HJ, Dias VT, Reckziegel P, Trevizol F, Dolci GS, Carvalho NR, Soares FAA, Rocha JBT, Emanuelli T, Bürger ME (2011) Exercise affects memory acquisition, anxiety-like symptoms and activity of membrane-bound enzyme in brain of rats fed with different dietary fats: impairments of trans fat. Neuroscience 195:80–88.

Trindade de Paula M, Poetini Silva MR, Machado Araujo S, Cardoso Bortolotto V, Barreto Meichtry L, Zemolin APP, Wallau GL, Jesse CR, Franco JL, Posser T, Prigol M (2016) High-fat diet induces oxidative stress and MPK2 and HSP83 gene expression in Drosophila melanogaster. Oxid Med Cell Longev 2016:4018157.

Vrablik TL, Watts JL (2013) Polyunsaturated fatty acid derived signaling in reproduction and development: Insights from *Caenorhabditis elegans* and *Drosophila melanogaster*. Mol Reprod Dev 80:244–259.

Yang Z, Bertolucci F, Wolf R, Heisenberg M (2013) Flies Cope with Uncontrollable Stress by Learned Helplessness. Curr Biol 23:799–803.

Zemdegs J, Quesseveur G, Jarriault D, Pénicaud L, Fioramonti X, Guiard BP (2016) High-fat diet-induced metabolic disorders impairs 5-HT function and anxiety-like behavior in mice. Br J Pharmacol 173:2095–2110.

Ziegler AB, Ménagé C, Grégoire S, Garcia T, Ferveur JF, Bretillon L, Grosjean Y (2015) Lack of dietary polyunsaturated fatty acids causes synapse dysfunction in the Drosophila visual system. PLoS One 10:1–15.

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroscience.2020.07.042.

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