the cell during each i-E curve recording. Decrease of anodic limiting current originating from HydroxoPerhydroxoMercury(II) complex [Hg(O2H)(OH)] formation in alkaline solutions of hydrogen peroxide at potential of mercury dissolution upon gradual addition of antioxidants was observed. The assay based on that decrease was optimized (Sužnjević *et al*., 2011) and applied on wide variety of food and biological samples (Gorjanović *et al*., 2013b). Dependence of decrease of HPMC anodic limiting current on volume or mass of gradually added complex samples has been followed and plotted. The slope of the starting linear part of that plot was considered as a measure of antioxidant (AO) activity. The activity was expressed as percentage of anodic limiting current decrease per volume or mass of complex samples added (%/mL or %/mg).

Based on the results, fruit/vegetable substrates could be arranged according to their antioxidant properties, from the highest to the lowest, in the following way: banana (0.169 ± 0.003%/mg) > tomato (0.108 ± 0.001%/mg) > carrot (0.073 ± 0.004%/mg) > apple (0.035 ± 0.001%/mg). We must emphasize that we always peel an apple before cooking the substrate. This could be an explanation for its low antioxidant activity, since the majority of compounds with high antioxidant potentials are found in the apple skin (Wolfe *et al*., 2003). Further, cooking destroys part of the AOs, so our results can not be compared to those obtained for fresh fruit. Nevertheless, information considering differences in antioxidant properties of aforementioned diets could be useful for further experimental purposes.

Acknowledgment: This work was supported by the Serbian Ministry of Education, Science and Technological Development, Grant No. 173012.

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Creation and standardization of methods for ethological analysis of *Drosophila melanogaster*: preference test and immobilization stress.

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Introduction

The many fundamental biological mechanisms conserved between *Drosophila* and *Homo sapiens* justify the study of *Drosophila* in many biological fields, including neurobiology of learning, memory, and stress (Jennings, 2011). As observed in different vertebrates, like *Rattus norvegicus*, *Mus musculus*, or *Danio* *rerio*, *Drosophila* react to uncontrollable and inescapable stress exposure with decreased escape or avoidance named as "learned helplessness" (Vollmayr and Gass, 2013). In this regard, a study showed that individuals of *Drosophila melanogaster* expressed "learned helplessness" when exposed to thermal shocks as "punishment" for stopping their locomotion for one second (Yang *et al*., 2013). The presence of "learned helplessness" in *Drosophila* indicates the existence of complex mechanisms in these organisms allowing the study of the neurobiology of stress (Vollmayr and Gass, 2013).

Additionally, chronic or acute stress is one of the environmental factors involved in the development of psychiatric disorders (Joca *et al.*, 2003). Preclinical studies aiming to characterize treatments for psychiatric disorders usually test the anti-stress properties of a substance in laboratory animals submitted to inescapable stress such as immobilization or forced swimming (Lino-de-Oliveira *et al*., 2001; Lino-de-Oliveira *et al*., 2005). Indeed, it was already observed that "learned helplessness" in *D. melanogaster* is reduced by previous treatment with lithium (Ries *et al*., 2017) indicating that, as in vertebrates, the response of flies to stress is ameliorated with the treatment with antidepressants. Together, present evidence indicates that *D. melanogaster* may be useful for the discovery of new psychotropic medications.

Studies on behavior and drug testing in vertebrate animals have been criticized in the field of ethics and animal welfare (Huet and Haan, 2014). Currently, there are initiatives aiming to decrease the quantity or replace the vertebrates, by invertebrates, for example, in biomedical studies. These initiatives are known as 3Rs: reduction, refinement, and replacement (Huet and Haan, 2014). Therefore, the present study is aimed to describe the behavior of *D. melanogaster* against different stressful stimuli targeting the standardization of its use in neurobiology of stress and antidepressants. For that, adult flies of *D. melanogaster* were bred in four palatable media and the behaviors of these animals were analyzed in two different situations: a test of preference for palatable food and an immobilization stress. Immobilization stress was used as inescapable stress in studies using rats (Ueyama *et al*., 1997; Lino-de-Oliveira *et al*., 2001). The hypothesis is that the flies will prefer the food where they were bred and, after the stress exposure, they will reduce their preference or the search for food.

Material and Methods

Behavioral experiments were performed using wild male and female *D. melanogaster*. The stocks were obtained from Stock Center Tucson (Arizona, U.S.A.), and they were maintained in the Drosophilids laboratory at the Federal University of Santa Catarina. The animals were maintained under natural temperature and lighting. The adults were removed from the stock of the laboratory and were bred in a medium made of corn. Initially, each glass contained five males and five females, and they were maintained in different media. These studies comprised three different pilots. In all of them, flies were submitted to tests of preference for palatable foods and immobilization stress.

In the first pilot, flies were bred in four different types of media: alcohol, banana, molasses, and complete media around seven days before the tests (Figure 1a). 24 hours before the tests, flies were put in a glass containing only agar medium (1%) for the food privation. The tests were realized in three consecutive steps (pre-stress, stress, and post-stress) to flies of each medium (Figure 1b). A Petri dish divided in four quadrants was used to test the preference of substrate. Three quadrants were filled with agar and one quadrant with palatable medium (Figure 1c). In the pre-stress step, after 24 hours of food privation, eight flies of each medium were placed in the Petri dish (n = 32). To put the flies in the Petri dish they were anesthetized with CO_2 and handled with tweezers. Before the next step, flies were anesthetized again. Only a male and a female of each medium were used in the stress and post-stress steps (n = 8). Other flies used in the pre-stress went back to the glass with medium. Immobilization stress was induced putting the flies into a hole made in agar in a 96-well plate for PCR (Figure 1b). The hole was made with a tube of 2 millimeters of diameter. The post-stress was done as the same way of pre-stress. All steps were filmed for 10 minutes.

Alcohol was selected as one of the bred medium, because it is known that drosophilids have adaptations to live in environments containing this substance. However, we observed that the concentration of alcohol used here (14%) was not adequate to create the animals because many flies died in this medium. Despite that they can survive in places containing ethanol, in natural environment flies can avoid places with concentration higher than 5% (Devineni and Heberlein, 2013). Therefore, this exposure can be intermittent in

a

Medium	Ingredients	Proportions
Complete medium	Water	79 mL
	Molasses	7 mL
	smashed banana-“caturra” type	6 g
	Alcohol	14 mL
	Agar	1 g
Banana	Water	75 mL
	smashed banana-“caturra” type	25g
	Agar	1 g
Molasses	Water	92.5 mL
	Molasses	7.5 mL
	Agar	1 g
Alcohol	Water	86 mL
	Alcohol	14 mL
	Agar	1 g

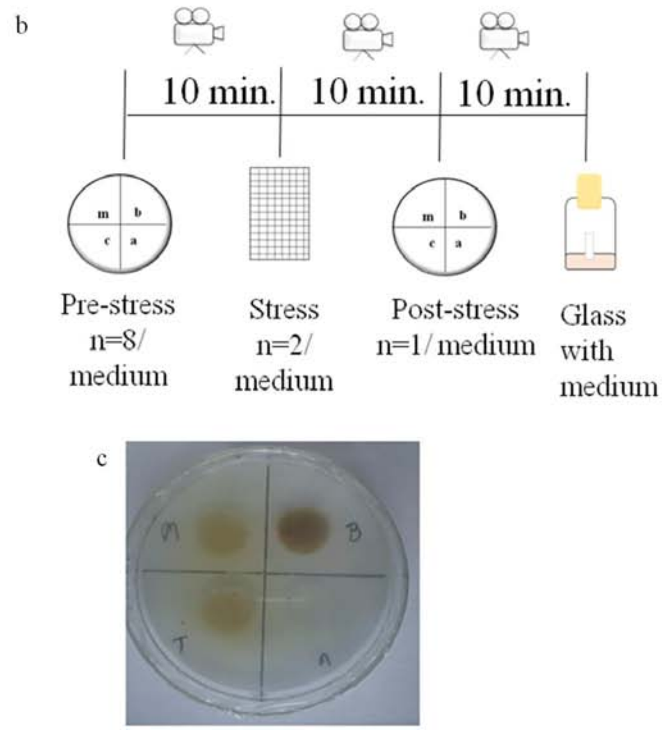


Figure 1. a. Ingredient proportions used in each medium. b. Timeline of the test. c. Petri dish used for the preference test.

contrast to laboratory conditions. The time and the method for food privation were not adequate, because many flies died in this period. Krashes and Waddell (2008) maintained flies without food for a period of 16 to 20 hours in a glass contained only a filter paper soaked in water. In the next pilots this methodology was used. The use of CO_2 and the handling of the flies with tweezers were also difficulties to maintain all the animals alive during the tests. Apparatus and methods used to observe the animal's behavior were a problem. Flies and medium in the Petri dish showed overlapping of colors and it did not allow a good visualization of the behaviors in the videos. The apparatus used in immobilization stress was not good, because the hole made in the agar was small in comparison to size of the flies. This hole had 0.2 cm of diameter and adults of *D. melanogaster* has 0.1 cm in length (Pitnick and García-Gonzalez, 2002). The locomotion of the flies and the withdrawal of the apparatus using tweezers became difficult. Besides this, flies attached on the agar, because did not have a protection “between” the agar and the animals.

In the second pilot, the flies were created in three types of media: banana, molasses, and complete medium, and they were maintained on this medium for two weeks before the tests (n = 10/medium) (Figure 2a). An apparatus made by students of the laboratory was used for the preference test. This apparatus was named “plus maze for preference test” and each tube of these maze contained 1.5 mL of the media: banana, molasses, complete medium, and agar (Figure 2c). The tests were also realized in three consecutive steps (pre-stress, stress, and post-stress). A female and male of each medium was used in the tests (n = 6). Before this, flies were deprived of food for 16 hours (Krashes and Waddell, 2008). They were placed into a glass containing a filter paper soaked in water (Figure 2b). For the preference tests (pre and post-stress), flies, individually, were placed into the plus maze and it was covered with a cover slip (Figure 2b). Immobilization stress was induced by putting the flies into a hole made in agar in a 96-well plate for PCR and this was made with a tube of 3 millimeters of diameter. All steps were filmed for 15 minutes (Figure 2b). To transfer the flies between different places, they were anesthetized with CO_2 and handled with tweezers. After five days, a retest was made using the same flies and following the same protocol. Behavioral catalogs were developed to the preference and to immobilization tests and after analysis of the films we observed that the size of the plus

a

Medium	Ingredients	Proportions
Complete medium	Water	79.5 mL
	Molasses	14.5 mL
	Smashed banana-“caturra” type	6 g
	Agar	1 g
Banana	Water	75 mL
	Smashed banana-“caturra” type	25 g
	Agar	1 g
Molasses	Water	92.5 mL
	Molasses	7.5 mL
	Agar	1 g

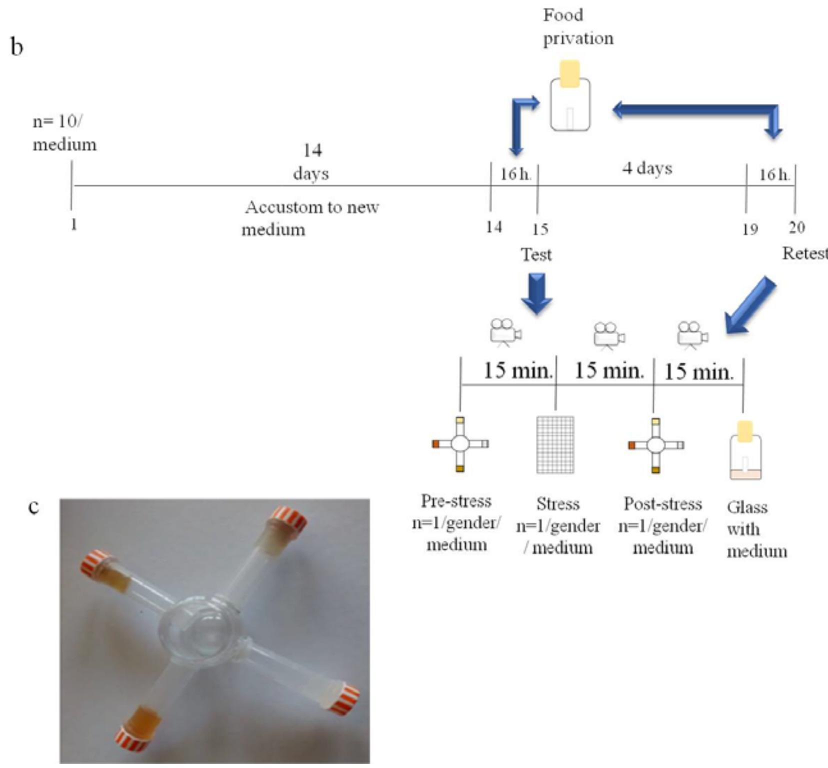


Figure 2. a. Ingredient proportions used in each medium. b. Timeline of the test and retest. c. Plus maze used for the preference test.

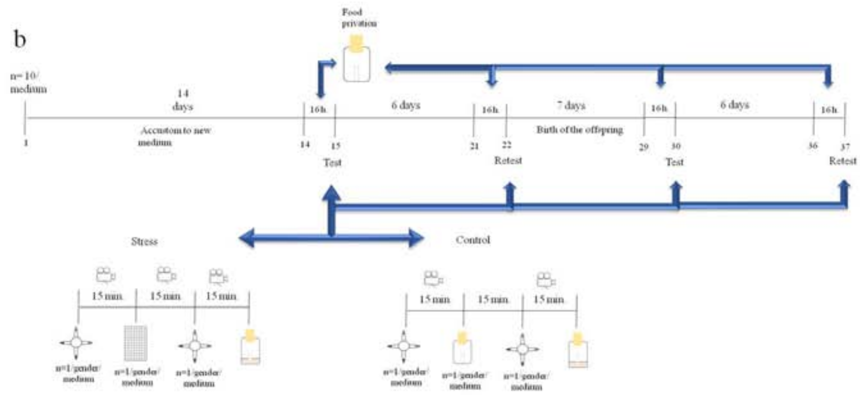
maze was not satisfactory, because the animals spent more time in the center of the apparatus and did not explore the arms with food. In behavioral studies with rats, for example, it is common to use a plus elevated maze. The size of these apparatuses agrees with our hypothesis. Pellow *et al*. (1985) used a plus elevated maze with arms of 50 cm in length for adult rats with 20 cm in length. The plus maze used in our study had arms with 4.5 in length. With the proportions used for rats, the flies used in this work would be 1.8 cm in length, however, adults of *D. melanogaster* are 0.1 cm in length (Pitnick and García-González, 2002). Das *et al*. (2016) said that starvation can contribute to the expression of memories associated to sugar. Among all the six animals tested on this pilot, immobility on the center of the plus maze was the behavior observed with more frequency in the videos. Three animals explored the arms of the plus maze, but this exploration did not occur in the pre- and post-stress steps; therefore, the arm exploration can be random. Using CO_2 to anesthetize the animals was another factor that would increase the time of immobility and decrease the plus maze exploration. This method to anesthetize is used in some studies with drosophilids (Lefranc and Bundgaard, 2000; Zimmerman *et al*., 2008), but the repeated exposure to CO_2 can influence the behavior of these animals. Immobilization stress can be another factor that decreased the locomotion of the flies, mainly during the post-stress steps. It is known that learned helplessness exists in *D. melanogaster* (Yang *et al*., 2013) and it can collaborate to reduce the locomotion and the looking for palatable food in the plus maze.

In the third pilot, flies were bred in four media: alcohol, banana, molasses, and complete medium (Figure 3a). They were maintained on these media for two weeks until start of the tests (n = 10/medium). Before the tests these flies were deprived of food for 16 hours, as in the second pilot (Figure 3b). To the preference test a small plus maze was created (Figure 3c). Flies were divided into two groups: control and stress. Two flies (male and female) of each medium and each group were tested (n = 16). For the stress group, the tests were performed in three consecutive steps: pre-stress, stress, and post-stress steps. Flies of the control group, on the second step, were placed into the glass for food privation (Figure 3b). On the pre- and

a

Medium	Ingredients	Proportions
Complete medium	Water	70 mL
	Molasses	9 mL
	Smashed banana-"caturra" type	6 g
	Alcohol	15 mL
	Agar	1 g
Banana	Water	70 mL
	Smashed banana-"caturra" type	30 g
	Agar	1 g
Molasses	Water	91 mL
	Molasses	9 mL
	Agar	1 g
Alcohol	Water	94 mL
	Alcohol	6 mL
	Agar	1 g

b



c

Figure 3. a. Ingredient proportions used in each medium. b. Timeline of the test and retest to stress and control groups. c. Plus maze used for the preference test.

post-stress steps, flies, individually, were placed into the plus maze and it was covered with a cover slip. The immobilization stress was made in the same way as the second pilot. After each couple generated offspring, a male and female of each medium/group were also tested (n = 16) following the same protocols used for their parental. All steps were filmed for 15 minutes (Figure 3b). To transfer the flies between the "house glasses" to the "privation food glass" they were anesthetized with CO_2 and handled with tweezers. To transfer them between the steps of the tests, an entomological aspirator was used. A polystyrene box was used to do the films with less light interference on the animal behaviors. After seven days, retest was made using the same flies and following the same protocol (Figure 3b). The new plus maze, besides not having the ideal size yet, was better than that used on the second pilot, because it was smaller than the first. Although animals still carry out behaviors of immobility, the exploitation of the arms of the plus maze was more expressive than that observed in the second pilot. Knowing that *D. melanogaster*, as other insects, had positive phototropism (Gao *et al*., 2008) we did the videos inside a polystyrene box. It was an improvement in relation to the other pilots, because the light interference on the behaviors of the flies was lower than in the first and second pilots. The use of the aspirator had pros, because the flies were not exposed many times to CO_2, and cons, because using the entomological aspirator was more difficult to maintain the animal in the center of the plus maze to begin the test. After analyzing the videos, it could be observed that the time spent in locomotion on the plus maze increased and less animals died during the tests, compared to other pilots. Barron (1999) suggested that behavior studies avoid CO_2 to anaesthetize the animals, because CO_2 can change the behavior of them. However, among the eight parental flies that were exposed to immobilization stress, six increased the time of immobility. It was observed that the arms of the plus maze were explored with more frequency than in the second pilot. The same animals reduced their arm exploration after stress steps. The environment that insect larvae and adults live in can influence behaviors, such as food preference, of these animals (Abed-Vieillard and Cortot, 2016). But, only 7 of 32 animals used in tests explored more often the arm containing the same food of their "house glass". *Drosophila* has a preference to concentrations up to 5% of ethanol, looking for places with odor or food containing this substance (Kaun *et al*., 2011). But, during the preference test, the arm containing alcohol medium did the flies explore the less. The other arms, containing media with sugar, were more explored. *D. melanogaster* could be good species to study anhedonia, but to confirm this hypothesis a larger sample number must be used in the behaviors tests. Beside this, a second method to stress flies could be efficient to obtain a better result, too.

The results of the three pilots of this study showed that *D. melanogaster* is sensitive to alcohol and avoids this substance or dies when in concentrations higher than 5%. Besides this, when in starvation, flies of this species shows preference for substrates that contain palatable food, such as sugar. Results also indicate that *D. melanogaster* is sensitive to stressful situations, like related to immobilization and CO_2 anesthesia, and these situations could modify their behaviors to looking for food. This study shows a preliminary analysis of behavior of *D. melanogaster*. It is important to establishment of models for replacement of vertebrates in behavioral studies. However, to confirm the hypothesis it is necessary for a greater behavior analysis, with higher sample number.

Acknowledgments: We thank the technician Marcos Antonio Loureiro for the help in breeding the flies and for all the instructions.

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Mapping *Drosophila* phototaxis behavior mutants; Possibly extend method to genetic diseases in human families.

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Recombination based mapping traditionally measures distances between traits or markers by recombination fractions in order to map them. An alternative recombination based approach, based on localizing crossovers in intervals defined by markers having known locations, efficiently positions traits, including those previously difficult to map, to a chromosome section between such markers. This approach makes use of the fact that many gametes will carry a chromosome with either no crossover or only a single crossover. The class of non-crossover chromosomes can be used to map a trait to a specific chromosome ("not independent assortment" of the trait with reciprocal parental gametes). The class of gametes with a single crossover, identified by a change in phase at only a single interval between known markers, divides the chromosome into left and right portions; the sum of such divisions, over several progeny with single crossovers, can be used to localize the trait to a specific interval. For discrete dichotomous traits, such an approach is significant with relatively few progeny. For quantitative, or polychotomous, or weakly penetrant traits, the reciprocal single crossover-gametes for an interval can be typed for systematic differences in