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The role of dopamine in *Drosophila melanogaster* Meigen, 1830 fitness under chronic stress

Роль дофамина в регуляции приспособленности *Drosophila melanogaster* Meigen, 1830 в условиях хронического стресса

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Key words: Chronic stress, *Drosophila melanogaster*, feeding behavior, fertility, longevity.

Ключевые слова: хронический стресс, *Drosophila melanogaster*, пищевое поведение, плодовитость, продолжительность жизни.

Abstract. Dopamine is a key link of the neuroendocrine stress response in insects, *Drosophila melanogaster* Meigen, 1830 in particular. Since earlier a decrease in the dopamine level had been found in insects having undergone chronic stress, we assumed that an artificial increase in the dopamine level can lead to increase insect resistance to chronic stress. Here we present the results of experimental testing of this assumption. It turned out that increasing the dopamine level by feeding *D. melanogaster* imagoes with its precursor L-DOPA does not elicit increased viability under acute stress but impedes it being increased as a result of chronic stress. It also did not affect *D. melanogaster* fertility both under normal conditions and under chronic stress. However, we discovered that increasing the dopamine level leads to decreased food consumption in flies under chronic stress. So, the data obtained allow us to conclude that dopamine takes part in controlling feeding behavior under chronic stress but does not regulate longevity and fertility.

Резюме. Дофамин является ключевым звеном нейроэндокринной стресс-реакции насекомых, *Drosophila melanogaster* Meigen, 1830 в частности. Поскольку ранее у насекомых, испытывавших хронический стресс, было обнаружено снижение уровня дофамина, мы предположили, что его искусственное повышение может способствовать большей устойчивости насекомых к хроническому стрессу. В данной работе представлены результаты экспериментальной проверки этого предположения. Оказалось, что повышение уровня дофамина путём кормления имаго *D. melanogaster* его предшественником L-ДОФА не приводит к повышению выживаемости при остром стрессе, зато препятствует её повышению, вызываемому хроническим стрессом. Оно также не оказало влияния на плодовитость *D. melanogaster* как в нормальных условиях, так и при хроническом стрессе. В то же время обнаружено, что повышение уровня дофамина приводит к снижению потребления пищи у мух в условиях хронического стресса. Таким образом, дофамин участвует в контроле пищевого поведения при хроническом стрессе, но не регулирует продолжительность жизни и плодовитость.

Introduction

Living beings respond to short-term and long-term stress differently. The response to acute stress is rather well-described in insects [Gruntenko, Rauschenbach, 2008]; however, much less is known about the consequences of chronic stress. Dopamine is a key part of the neuroendocrine stress response in adult insects, including *Drosophila melanogaster* Meigen, 1830, and influences insect survival [Gruntenko et al., 2004; Ueno et al., 2012; Hanna et al., 2015]. It is known that at each point in time, dopamine content in *D. melanogaster* is determined by its synthesis/degradation ratio; the intensity of the synthesis depends on the pool of its precursor, tyrosine [Wright, 1987]. Tyrosine content, in turn, depends on the activity of alkaline phosphatase (ALP), which converts inert tyrosine conjugate, tyrosine-O-phosphate, into tyrosine [Wright, 1987]; the main pathway of dopamine inactivation in *D. melanogaster* is N-acetylation by dopamine-dependent N-acetyltransferase (DAT) [Dempsey et al., 2014]. So, changes in the activity of these enzymes of dopamine metabolism can serve as markers of changes in amine content. Earlier we showed that daily short-term heat stress (38 °C, 1 h) caused increased activity of both enzymes, ALP and DAT [Burdina et al., 2019]. Under such chronic stress, the following changes in fitness and metabolism of adult flies were observed: longevity, fertility, fly weight and lipid content were significantly decreased; at the same time, carbohydrate content and survival under acute heat stress were increased compared to the control group [Gruntenko et al., 2021]. Based on these data, we decided to try and correct the negative consequences of chronic stress (38 °C, 1 h daily) on adult *D. melanogaster* fitness and metabolism through experimentally raising their dopamine level by feeding the with the dopamine precursor, L-DOPA, which, as shown by us earlier [Bogomolova et al., 2010], causes an increase in dopamine content.

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Materials and methods

Drosophila melanogaster strain and heat stress modes

Flies of the wild type *Drosophila melanogaster* strain Canton S were maintained on a standard medium (agar-agar, 7 gL−1; corn grits, 50 gL−1; dry yeast, 18 gL−1; sugar, 40 gL−1) at 25 °C under a 12:12h light:dark cycle, and the adults were synchronized at eclosion (flies were collected every 3–4h). Starting from the second day, experimental groups were exposed to mild heat stress (38 °C, 1h) daily. The control groups were maintained at 25 °C. In the fertility and longevity experiments, flies were maintained at 25 °C until the end of their reproduction period or life. In all the other experiments, flies were analyzed at two weeks of age. In the fertility experiments, flies were transferred to a fresh medium daily, and in all other experiments, three times a week.

L-DOPA feeding

Dopamine content was modified by feeding the flies with the dopamine precursor L-dihydroxyphenylalanine (L-DOPA, «Sigma»), which resulted in a twofold rise in dopamine level [Bogomolova et al., 2010]. In experiments on fertility and longevity, 1-day-old flies were placed in test tubes (five females and five males per test tube) with a yeast-free nutrient medium, to which a solution containing 2 % yeast and 1 % L-DOPA was added (control flies did not receive L-DOPA). Flies were maintained under these conditions until the end of the reproduction period or the end of their life, respectively. In all other experiments, feeding with L-DOPA was carried out in a similar way, but was limited to the last two days of the experiment; before that, flies were kept on a standard nutrient medium.

Fecundity and longevity analysis

For fecundity analysis, five newly eclosed females and five males were placed into a vial (10 vials per group) and were transferred to vials with fresh medium daily. Fecundity is presented as number of offspring per female per 24 h. For longevity analysis, five newly eclosed females and five males were placed into a vial (10 vials per group) and were transferred to vials with fresh medium three times a week.

Capillary feeding assay

Changes in feeding were evaluated using the method of Ja et al. [Ja et al., 2007] modified by Williams et al. [Williams et al., 2014]. Five 12-day-old females of each group were placed in a 10 cm x 2 cm (height x diameter) vial containing 1 % agarose (5 cm high) which provided humidity for the flies during the experiment. A capillary glass tube (Narishige, Japan) was filled with 15 µl of liquid food, which contained 5 % sucrose and 5 % yeast extract, and was stuck into a vial stopper. The initial food level in the capillary tube was marked and 0.1 µl of mineral oil was used to prevent random evaporating. The vials (16 for each group) were kept in a 25 °C incubator for 24 h, and then the final food level in the capillary tube was marked to determine total food intake per day. A «blank» vial without flies was used to detect the rate of food evaporation. The average feed consumption of a fly was calculated by dividing the total food intake by the number of flies in the vial, minus the «blank» value.

Statistical analysis

The data on fertility (number of progeny per female per day) were analyzed via two-way mixed-design ANOVA (with day after eclosion as the within-subjects factor and stress as the between-subjects factor). The data on feed consumption, longevity and survival under acute heat stress were analyzed via one-way ANOVA (with stress as the between-subjects factor). Dann's stepwise post hoc test was used to compare group means in ANOVA. The results were considered significant at probability level < 0.05.

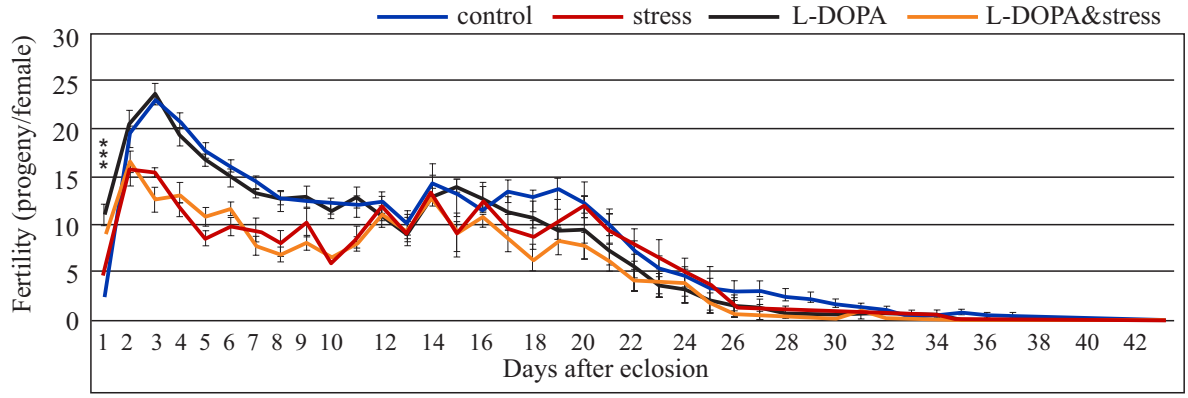
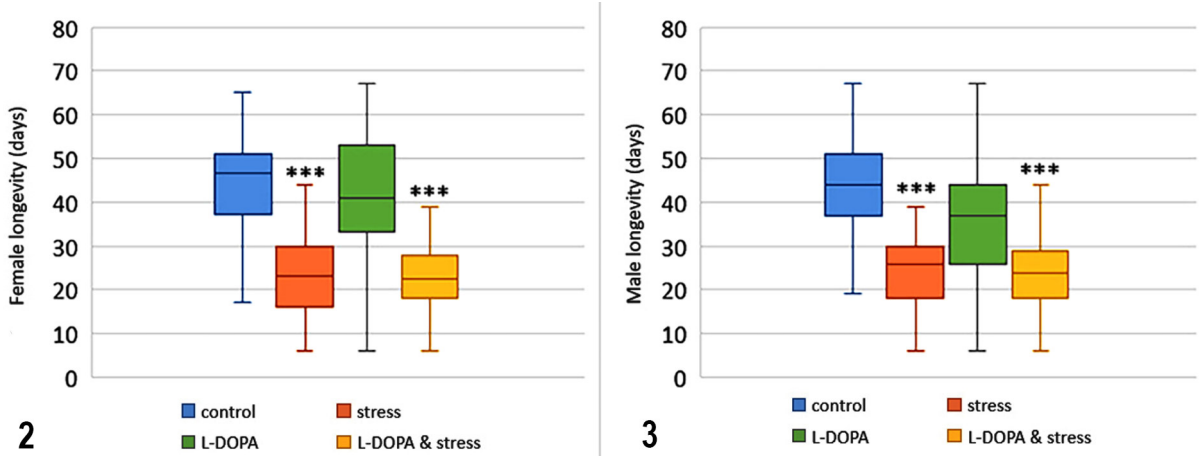


Fig. 1. The effect of chronic stress (38 °C, 1 h, daily), L-DOPA feeding and the combination of these two factors on *D. melanogaster* fertility. Each value is an average of 10 tests. Means ± SEM. The asterisk indicates significant differences between L-DOPA-fed and control flies during the first day of oviposition. Three asterisks, p < 0.001, Dann's stepwise post-hoc test.

Рис. 1. Влияние хронического стресса (38 °C, 1 час, ежедневно), кормления L-ДОФА и комбинации этих двух факторов на плодовитость *D. melanogaster*. Данные представлены как среднее значение ± SEM, n=10. Звёздочка — достоверные различия между мухами, получавшими L-ДОФА, и контрольными в течение первого дня откладки яиц. Три звёздочки, p < 0,001, post-hoc тест Данна.



Figs 2–3. The effect of chronic stress (38 °C, 1 h, daily), L-DOPA feeding and the combination of these two factors on the longevity of *D. melanogaster* females (A) and males (B). 43–50 flies per group; means ± SEM. The asterisk indicates significant differences between stressed and unstressed groups. Three asterisks, p < 0.001, Dann's stepwise post-hoc test.

Рис. 2–3. Влияние хронического стресса (38 °С, 1 час, ежедневно), кормления L-ДОФА и комбинации этих двух факторов на продолжительность жизни самок (А) и самцов (В) *D. melanogaster*. Данные представлены как среднее значение ± SEM, n = 43–50. Звёздочка — достоверные различия между группами, подвергавшимися и не подвергавшимися стрессу. Три звёздочки, p < 0,001, post-hoc тест Данна.

Results and discussion

Dopamine and chronic stress effects on *D. melanogaster* fertility

In order to find out if dopamine could rescue *Drosophila melanogaster* fitness under chronic stress, we have studied the fertility level of wild type flies of the Canton S strain under the effect of two factors and their combination: chronic stress (38 °C, 1 h daily) and L-DOPA feeding leading to the dopamine level increase (Fig. 1). We found out that the dopamine level increase resulted in fertility being increased on the first day of oviposition only (L-DOPA – $F_{(1, 39)} = 45832.00$, $p < 0.001$, ANOVA). In the following days, regardless of L-DOPA treatment, chronic stress led to a stable decrease in fertility (L-DOPA – $F_{(1,1719)} = 3.89$, $p = 0.08$, stress – $F_{(1,1719)} = 31.78$, $p < 0.001$, ANOVA). Thus, dopamine failed to rescue *D. melanogaster* fertility under chronic stress.

As to the fertility increase in the L-DOPA-fed flies on the first day, we believe that it is associated with the stimulating effect of dopamine on the juvenile hormone (JH) level, which was shown previously for young *D. melanogaster* females [Gruntenko, Rauschenbach, 2008], and the JH level increase being necessary for the start of oogenesis [Soller et al., 1999]. At the same time, in mature and actively reproducing flies, dopamine has the opposite effect on JH [Gruntenko, Rauschenbach, 2008], but, apparently, being long-term, this effect is compensated by mechanisms maintaining hormonal homeostasis and does not significantly influence fertility in either the stressed or non-stressed group of flies.

Dopamine and chronic stress effects on *D. melanogaster* longevity

Another significant life trait, lifespan, was next to be studied. We have estimated the longevity of Canton S flies of both sexes under (i) chronic stress (38 °C, 1 h daily), (ii) L-DOPA feeding and (iii) combination of these two factors (Figs. 2, 3). Chronic stress caused a strong decrease in the longevity of both females (Figs 2–3) and males (Figs 2, 4), regardless of L-DOPA feeding ($p < 1 \cdot 10^{-7}$, Dann's stepwise post-hoc test). However, the increase in dopamine level did not seem to have any effect on either male or female lifespan. So, dopamine appears not to be involved in the lifespan regulation both under normal and chronic stress conditions.

It should also be noted that although dopamine is a well-known participant of the neuroendocrine response to acute stress, its pharmacological increase failed to

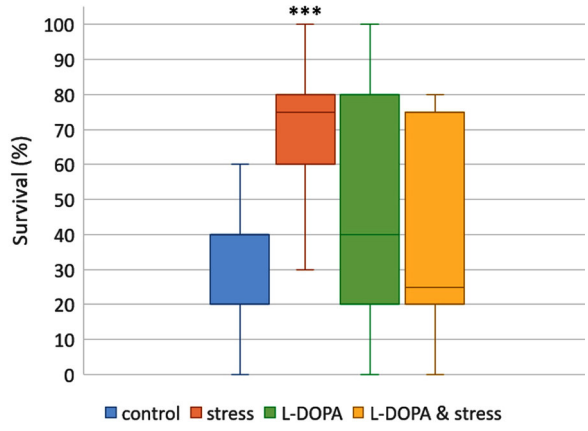


Fig. 4. The effect of chronic stress (38 °C, 1 h, daily), L-DOPA feeding and the combination of these two factors on *D. melanogaster* survival under acute heat stress (38 °C, 4 h). Each value is an average of 30 tests. Means ± SEM. The asterisk indicates significant differences of «stress»-group from all other groups. Three asterisks, p < 0.001, Dann's stepwise post-hoc test.

Рис. 4. Влияние хронического стресса (38 °С, 1 ч, ежедневно), кормления L-ДОФА и комбинации этих двух факторов на выживаемость *D. melanogaster* при остром тепловом стрессе (38 °С, 4 ч). Данные представлены как среднее значение ± SEM, n=30. Звёздочка — достоверные отличия группы «стресс» от всех остальных групп. Три звёздочки, p < 0,001, post-hoc тест Данна.

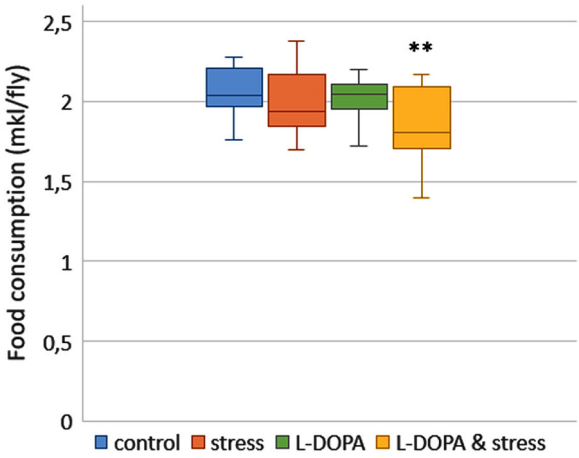


Fig. 5. The effect of chronic stress (38 °C, 1 h, daily), L-DOPA feeding and the combination of these two factors on food consumption in *D. melanogaster* females. Each value is an average of 16 tests. Means ± SEM. The asterisk indicates significant differences of «L-DOPA & stress» group from control and «L-DOPA» groups. Two asterisks, $p < 0.01$, Dann's stepwise post-hoc test.

Рис. 5. Влияние хронического стресса (38 °C, 1 ч, ежедневно), кормления L-ДОФА и комбинации этих двух факторов на потребление пищи самками *D. melanogaster*. Данные представлены как среднее значение ± SEM, n=16. Звёздочка — достоверные различия между группой «L-ДОФА и стресс», контрольной группой и группой «L-ДОФА». Две звёздочки, $p < 0{,}01$, post-hoc тест Данна.

prevent fertility and longevity loss occurring in *D. melanogaster* under chronic stress.

Dopamine and chronic stress effects on *D. melanogaster* survival under acute heat stress

It was shown earlier that moderate stress can increase the resistance to acute heat stress in *D. melanogaster* [Khazaeli et al., 1997; LeBourg et al., 2001; Hercus et al., 2003]; we also observed this effect following heat exposure (38 °C, 1 h), repeated daily for 2 weeks [Burdina et al., 2019]. An increase in the activity of the dopamine metabolism enzymes, ALP and DAT, was also found following such chronic stress [Burdina et al., 2019], which allows us to assume that the latter decreases dopamine level in flies. This assumption agrees well with the fact that *D. melanogaster* and *D. virilis* females with increased dopamine content have lower resistance to acute heat stress [Hirashima et al., 2000; Gruntenko et al., 2004]. Here we confirmed that chronic stress (38 °C, 1 h, daily, two weeks) increased survival under acute heat stress (38 °C, 4 h) in *D. melanogaster* females of wild type strain Canton S ($p < 6{,}3 \cdot 10^{-6}$, Dann's stepwise post-hoc test; Fig. 4). On the other hand, L-DOPA feeding (resulting in an increase in dopamine level [Bogomolova et al., 2010]) in females exposed to chronic stress levels out their survival under acute heat stress to that of unstressed groups, which corresponds to the data on negative correlation of dopamine content and acute heat stress resistance [Hirashima et al., 2000; Gruntenko et al., 2004]. However, no decrease in survival was observed under acute stress in the L-DOPA-fed group, which was not exposed to chronic stress. One of possible explanations for this fact could be an age-related decline in *D. melanogaster* stress resistance, demonstrated earlier [Belyi et al., 2020]. The authors exposed flies to various types of stress (starvation and infection with a pathogenic fungus) at different ages and found a strong negative correlation between age and stress resistance. So, it cannot be ruled out that the survival under acute heat stress in two-weeks-old flies is decreased and additional dopamine can't lower it any further.

Dopamine and chronic stress effects on *D. melanogaster* feeding behavior

One more advantageous life trait studied here is feeding behavior, estimated using a capillary feeding assay [Ja et al., 2007; Williams et al., 2014]. Food consumption was compared between four groups of the two-weeks-old *D. melanogaster* females: exposed to chronic stress (38 °C, 1 h daily); L-DOPA feeding during two days prior experiment; chronic stress and two-day L-DOPA feeding; control (Fig. 5).

It is notable that neither chronic stress itself nor L-DOPA feeding itself affected food consumption but the combination of these two factors resulted in its decrease ($p < 0.01$, Dann's stepwise post-hoc test). It seems that internal energy resources are not sufficient to cope with such a double load.

Living beings respond to short-term and long-term stress differently. Some hide and wait out the danger, while others flee or fight. We previously found that acute and chronic stresses have different effects on dopamine level and survival in *D. melanogaster* imagoes, reflecting different survival strategies used by living organisms under various stresses. Here we showed that a pharmacological increase in dopamine level during chronic stress resulted in changes in feeding behavior, which, together with our previous data on the dopamine ability to reduce glucose and trehalose levels, may indicate that energy metabolism is also affected. It is possible that dopamine is the trigger that switches an insect's survival strategy under various adverse conditions.

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