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Short Communication

Effect of antidepressant drug Flupentixol-Melitracen on locomotory behaviors of *Drosophila*

Azizul Islam Barkat, Rabeya Bosri Mehe Jabin, Farhin Momtaz Riana, Sumaiya Akter, Md Shamsudduha and Mohammad Shamimul Alam*

Genetics and Molecular Biology branch, Department of Zoology, University of Dhaka, Dhaka, Bangladesh

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ABSTRACT

Antidepressant drugs are frequently used in the treatment of depression. Their effects on locomotory behavior have been poorly studied in human and *Drosophila* models. In this study, the effects of an antidepressant drug on larval crawling, negative geotaxis, and TOR gene expression were investigated in a *Drosophila* model. Drug-treated flies showed significantly higher activity in the negative geotaxis assay. However, no statistically significant differences were observed in the other behavioral assays or in TOR gene expression.

Introduction

Depression, a mental disorder, is characterized by despair, pessimism, and a loss of interest in once-enjoyed pursuits. The prevalence of depression is increasing worldwide (Liu et al., 2020). The combination of flupentixol (0.5 mg) and melitracen (10 mg) drug is widely used as an antidepressant to treat clinical depression. Flupentixol acts as an inhibitor of D2 receptors, limiting dopamine activity in the brain, while melitracen blocks the reuptake of serotonin and norepinephrine at presynaptic terminals in neurons (Wang et al., 2015). The pathophysiology of depression might be influenced by the dysregulated TOR (Target of Rapamycin) pathway. It is found that the antidepressant increases the expression of the TOR gene, suggesting its involvement in regulating the TOR pathway (Réus et al., 2015). Antidepressant drugs have been associated with movement disorders (Revet et al., 2020). Therefore, it has the potential to disrupt motor function. *Drosophila* is regularly used to study motor function through larval crawling and negative geotaxis tests. The negative geotaxis assay utilizes the innate escape response of flies to move against gravity when agitated (Ali et al., 2011). Although Flupentixol-Melitracen is widely prescribed, its role in motor function remains poorly studied. In this study, we investigated the effects of antidepressant drugs on larval crawling, negative geotaxis, and TOR gene expression in the *Drosophila* model.

Wild fruit flies were collected from the local environment and reared on a standard food medium at 25°C under a 12:12 light:dark photoperiod. Morphological and molecular analyses (NCBI accession no. PQ198554) confirmed the species as *Drosophila ananassae*. The flies were then grouped into control and treatment. The control group was reared in standard food, while the treatment flies were cultured in drug-treated food. The antidepressant drug Frenxit (Beximco Pharmaceuticals Ltd., Bangladesh), was added to the treatment food following a previous study (Kruger and Denton, 2020). The dose applied in this experiment was 400 mg/L food, which is the LC_{50} (Lethal Concentration 50%) of the drug for the fly (Riana, 2024).

*Corresponding author:<shamimul@du.ac.bd>

For the larval crawling assay, the 3rd instar larvae from the two groups were gently transferred from the vials and washed off the food material from their bodies using a 5% sugar solution. A 2% agar plate was prepared to provide a smooth surface for crawling, and the larva was placed in the center. The agar plate was placed on a grid (1x1 cm) of paper (Fig.1), and a video camera was placed above the plate. One larva was placed on the agar plate at a time, and a 30-seconds video was recorded. For both groups, ten videos were taken (10 biological replicates). Three data types were taken for the crawling assay: bending, turning, and distance. A larva was regarded as turning when it moved less than 30 degrees and bending when it moved more than 30 degrees. The distance was determined by the number of grids it passed.

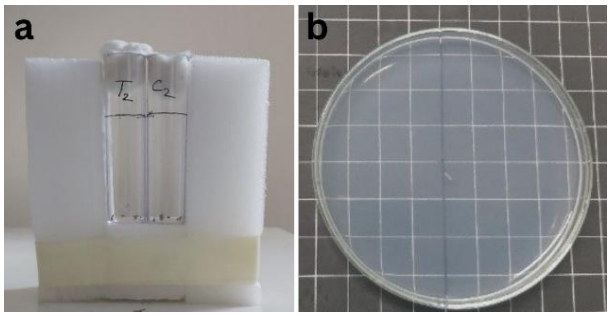

Fig. 1. *Drosophila* behavioral assay setup. a) Negative geotaxis: the number of flies crossed the mark from the bottom was observed. b) Larval crawling assay: an individual larva is dropped at the center of the plate to observe and image the locomotion.

To conduct the negative geotaxis assay, adult virgin flies were collected via cold anaesthetization on the day of emergence before they fully developed to mate. The assay was performed 1 hour after anaesthetization to ensure the flies had fully recovered from the anesthesia stress. The flies were sorted into control and treatment groups, with 10 flies per vial. Two vials for the two groups were marked at a vertical distance of 6 cm from the bottom (Fig. 1). The flies were tapped down, and the number of flies that climbed above the mark by 10 seconds was counted. The assay was repeated ten times, and the number of flies passing the mark was recorded as a percentage of the total number of flies.

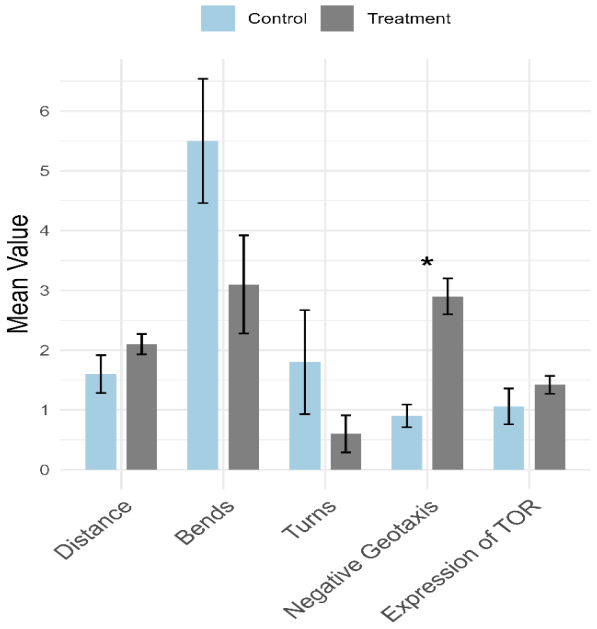

Fig. 2. Effect of antidepressant on *Drosophila*'s behavioral phenotypes and gene expression. Only the negative geotaxis behavior was statistically significant (p=0.0001) between the control and treatment flies. The other behavioral assays, i.e. distance, bends, turns and gene expression levels, were not statistically significant difference (p<0.05).

To measure TOR gene expression, a dye-based qPCR was used. After RNA extraction (Tiangen, China) from 10 3rd - instar larvae of *Drosophila melanogaster* Hikon-H strain, cDNA was synthesized according to the manufacturer's instructions (ABclonal, USA). Three biological replicates were used from each group, and each reaction was run in triplicate in qPCR. The total reaction cycle was 35 with the thermal conditions of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 60°C for 20 s. The RPL11 (Ribosomal Protein Loci 11) was taken as a housekeeping gene. The sequence of the primer was 5´-ACTTCGGTTTCGGCATCCA-3' (forward) and 5'- TTGCGCTTCCTGTGGTTCA-3' (reverse). The sequences of primers for the amplification of the TOR gene used in this study were 5´-CGAAAGCGTGATGCTGGTG-3' (forward) and 5'-

TTGCACTTGACCCGCTTGAT-3' (reverse). The $2^{-\Delta\Delta CT}$ Method was followed to analyze the expression of the targeted gene (Livak and Schmittgen, 2001).

To identify the significant differences between the groups, the t-test was used in this study. All the statistical analyses were conducted in excel and the graph was produced in R.

The larvae in both groups showed no significant difference in the larval crawling assay. The average distance the drug-treated and control group larvae crossed was 2.10 and 1.562 cm, respectively (p=0.1907). The average number of bends and turns in the control and treatment groups were 5.5 and 3.1 (p=0.086) and 1.8 and 0.6 (p=0.21) (Fig. 2).

Significant differences in the negative geotaxis assay were observed between the groups. The average number of individuals (n=10) crossing the '6 cm' mark in 10 seconds was 0.9 (9%) in the control group and 2.9 (29%) in the treatment group. The p-value is 0.0001. The flies of the treatment group exhibited a greater capacity to respond to a stimulus than those of the control group.

The TOR gene was expressed slightly higher in the treatment group. TOR The expression was 1.42- and 1.06-fold higher in the treatment and control groups, respectively. Though the expression of TOR was higher in treatment, it was not statistically significant (p=0.722) (Fig. 2).

According to this study, antidepressant drugs cause changes in the negative geotaxis behavior of *Drosophila*. The drug-treated flies show high locomotion activity. The TOR gene was expressed at a higher level in the treatment group's larvae, though the difference was not statistically significant. When the TOR gene is downregulated, behavioral deficits are observed (Potter et al., 2019). Based on the present findings, we hypothesize that higher expression of the TOR gene may potentially enhance geotaxis activity. However, the current study, with its small sample size, is not sufficient to establish a definitive hypothesis. Further study is required to analyze the expression pattern of TOR in drug-treated pupae and adult fruit flies with a large sample size.

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Authors contribution

Azizul Islam Barkat: concept development, laboratory activities, manuscript preparation, review and editing; Rabeya Bosri Mehe Jabin: concept development, laboratory activities; Farhin Momtaz Riana: concept development, laboratory activities; Sumaiya Akter: laboratory activities, manuscript preparation; Md Shamsudduha: laboratory activities, manuscript preparation; Mohammad Shamimul Alam: concept development, review and editing, fund acquisition.

Conflict of interest

There is no conflict of interest regarding the publication.

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