# Suppressive and Enhancing Effects of 5-HT Agonist, Fluoxetine, and 5-HT Antagonist, Ondansetron, Respectively, on Food Competition in Rats

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**Abstract**

**Background and aim of the work:** Reduced brain serotonin (5-HT) activity was noted to be associated with increased aggression in animals, and vice versa. The current study aimed to investigate the effects of 5-HT agonist and antagonist, fluoxetine and ondansetron, respectively, on the competitive behavior of rats and their brain 5-HT levels.

**Methods:** Forty male albino rats were housed as pairs. All rats were food-deprived 23 hours/day. Based on competition results, all pairs of rats were classified into stable and unstable pairs, according to their success or failure in maintaining their social ranking. Stable pairs of rats were divided into 2 halves and 4 treatment groups. In the first half, stable winners and partner losers were treated with fluoxetine (FLX) (10 mg/kg body weight, intraperitoneal, i.p) or ondansetron (OND) (1 mg/kg body weight, i.p), respectively, and vice versa in the second half. Unstable pairs of rats that failed to retain their social ranking formed an additional treatment group. The effect of treatment with fluoxetine (10 mg/kg body weight, i.p) or ondansetron (1 mg/kg body weight, i.p) on inducing a stable social ranking was investigated. At the end of the study, using Elisa, 5-HT concentrations were determined in brains extracted from winners and losers of all treatment groups.

**Results:** Fourteen out of twenty pairs of rats showed a stable hierarchy. FLX treatment of winners produced a significant

reduction in their competition scores; whereas OND produced a significant increase in the competition scores of treated losers. Similarly, FLX depressed the competitive behavior of the previously unstable rats to become stable losers; OND could enhance the competitive behavior of the previously unstable pairs of rats to retain the stable winner position. OND has shown a depressive effect on the serotonin levels in the brains of all OND-treated rats. In contrast, serotonin levels were elevated in the brains of all FLX-treated rats.

**Conclusion:** FLX and OND had depressive and enhancing effects, respectively, on competitive behavior of rats. The rats' brain 5-HT levels were inversely related to the competitive behavior of rats.

**Keywords:** Fluoxetine, ondansetron, social hierarchy, serotonin.

## Introduction

The lives of humans and other species are greatly affected by their rank in a specific social hierarchy when living in groups ***(Zhou et al., 2018)***. The aggressive interaction among the groups of animals may determine the rank order of each animal in its social group ***(Olivier, 2005).*** This agonistic behavior of animals is derived by the limited resources of food, water, territory or sexually receptive females ***(Zhou et al., 2018)***. Animals with higher rank in their groups may have first access to the aforementioned limited resources. Indeed, once rank order is established it may decrease fighting between animals and save energy ***(Sapolsky, 2004).*** Several researchers have presented different animal models to evaluate the agonistic and competitive behavior of animals; social hierarchy and rank order of animals could be determined in these animal models, for example, modified food competition test. In this model, dyads of rats competed for a palatable food pellet; the authors observed successful winners and failing losers ***(Costa et al.; 2021).*** Several authors reported different animal models for competition, including sucrose solution competition test, water competition test, and tube test ***(Timmer et al., 2011; Lozano-Montes et al., 2019; Costa et al., 2021).***

It was important to investigate the central neural mechanisms that determine the rank order of animals and their social hierarchy. It was found that serotonin (5-HT) is a major determinant factor of the aggressive behavior of animals ***(Alekseyenko & Kravitz, 2014).*** Many authors reported that increased activity of brain 5-HT is accompanied by an inhibitory state of the brain ***(Daw et al., 2002)***, which may result in decreased aggression of that animal ***(Veenema, 2009).*** On the contrary,

decreased serotonergic activity in the brain is accompanied by an increased aggression ***(Márquez et al., 2013).***

Many studies have been conducted to investigate the effects of different drugs on the aggressive behavior of animals. Reduced aggression was observed in animals treated with 5-HT agonists. This depressive effect on aggressive behavior has been observed after treatment with either direct 5-HT receptor agonists or indirectly acting agonists that increase synaptic level of 5-HT by inhibiting its reuptake, e.g., fluoxetine ***(Odore et al., 2020)***, In contrast, 5-HT3 receptor antagonists (e.g., Ondansetron) have been reported to decrease 5-HT brain activity and increase aggression ***(Engleman et al., 2008)***. Therefore, the aim of the current study was to investigate the effects of fluoxetine and ondansetron on the competitive behavior of male albino rats; further, to explore the effects of these drugs on the serotonergic activity in the brains of rats competing in an animal model for food competition.

## Material and Methods

### Chemicals and Drugs:

Fluoxetine hydrochloride, obtained from Egyptian International Pharmaceutical Industries Company (EIPICO); 10 ml of Fluoxetine HCl solution was daily freshly prepared by adding 100 mg of Fluoxetine HCl to 1 ml of Ethanol 96% and 9 ml of sterile water for injection.

Ondansetron (Zofran® ampoule 4mg/ 2ml), obtained from GlaxoSmithKline (GSK), Zofran ampoule solution was used without dilution, the dose was calculated according to the body weight of the rat as a fraction of ml, the average injected volume was 0.2 ml zofran solution.

Sterile water for injection, ethanol 96%, and sterile normal saline (0.9% Na Cl), were obtained from Nasr Company for Pharmaceutical Industries.

### Methodology and Study Design:

### Experimental Animals

Forty male adult albino rats with initial body weight of 150-200g were obtained from organization of biological products and vaccines (Vacsera, Egypt). The rats were randomly assigned to 20 pairs of rats; these were housed as fixed pairs throughout the study; further, according to the competition scores of these animals, they were classified 2 groups, stable and unstable groups. They were kept at 25 ± 3°C, in a normal light-dark cycle throughout the study. Food and tap water were available ad libitum for a one-week acclimation period. Tap water was also available ad libitum during the remainder of the study. Then the animals were randomly assigned to pairs, i.e., they formed twenty pairs of rats and housed as fixed dyads for the remainder time of the study. During that time, the animals were food-deprived for 23 hrs./day. Subject body weights were

maintained fairly stable by restricting daily access to food to one hour (17:00-18:00 hr) after the experimental sessions for that day were concluded. The experimental work was performed according to the ethical approval number 202204MAL.

**Competition Sessions and Rank Assessment of Animals**

Food-deprived rats were trained to approach the food hopper and to consume 200 mg sucrose pellets whose signaled delivery occurred at 30 sec intervals; A glass funnel was used to deliver the individual food pellet whose signaled delivery created by the sound of hitting the glass funnel with a glass rod. Ten sucrose pellets were delivered per session. Rats were individually trained daily in a specified cage for training, one session per day, for seven days and immediately returned to their home cage at the end of the session ***(Costa et al.; 2021).*** Following completion of training, each rat of the dyad was coded with a specific color using food coloring (red or blue) applied to the tail base (twice a week) to easily distinguish each rat within its dyad. Two feeders full of regular rat food were supplied during the daily one-hour feeding to enhance food availability to all subjects. The subjects were housed as fixed dyads throughout the study to enhance the establishment of stable dominance rankings.

During competition sessions, the rat actively attempted to snatch the pellet, i.e., the animal attempts to physically displace the other animal in the process of gaining access to the pellet, then the rat ingested the pellet. The competition was scored by direct observation using a scoring sheet. Session scores were calculated by adding up the points for each individual session for each rat. A rat was ranked as winner (W) or loser (L) based on its composite score within its specific dyad. Before drug effects were determined, competition for sucrose pellets was monitored among the two rats of each dyad to determine the relative competitive performance and rank-order of each rat in its specific dyad ***(Costa et al.; 2021).*** Stability of rank order was achieved when the animal maintained the same rank position within its specific dyad for five sessions or more out of seven daily sessions per week, the animal is considered “winner”, and its partner animal is considered a “loser”. Pretreatment rank-order was determined based on the average scores of seven daily sessions. All dyads were classified into stable dyads and unstable dyads. Stable dyads could maintain their same rank order for at least five sessions out of seven. Unstable dyads failed to maintain the same rank order for five sessions out of seven. The effects of treatment with fluoxetine HCl (10 mg/kg body weight, intraperitoneal, i.p.) and ondansetron (1 mg/kg b.wt, i.p) on

competition scores of these dyads were investigated.

**Treatment Schedule**

After the pretreatment competition sessions, the stable dyads were determined; Fourteen out of nineteen dyads could maintain a stable hierarchy, and five dyads showed unstable hierarchy; one rat died, and its dyad has been excluded from the study. The fourteen stable dyads have been divided into two treatment groups; the first treatment group included 7 dyads in the range of cage number 1-12; whereas the second treatment group included 7 stable dyads in the range of cage number 13-20. The unstable dyads included 5 dyads.

In the first week of treatment, winners of the first group (#1-12) were treated with fluoxetine HCl (10 mg/kg b.wt., i.p) ***(Moskaliuk et al., 2022)*** whereas their corresponding partner losers were kept untreated, injected with 0.3 ml normal saline solution. In the second treatment group, winners were treated with Ondansetron (1 mg/kg b.wt, i.p) ***(Sumaya et al., 2016)***; whereas their corresponding losers were kept untreated, injected with 0.3 ml normal saline solution, i.p. In the unstable dyads, the blue rat was selected randomly (because we have neither winners nor losers at this stage) to be treated with Fluoxetine HCl (10 mg/kg b.wt., i.p), the other animal was kept untreated, injected with 0.3 ml normal saline solution, i.p. All treatments have been administered half an hour before testing the competition. The competition scores of all rats have been measured in seven daily sessions over a week period.

In the second week of treatment, losers of the first group have been treated

with ondansetron (1 mg/kg b.wt, i.p); whereas their corresponding partner winners were kept untreated, injected with 0.3 ml normal saline solution, i.p. In the second treatment group (#13-20), losers were treated with fluoxetine HCl (10 mg/kg b.wt., i.p); whereas their corresponding winners were kept untreated, injected with 0.3 ml normal saline solution, i.p. In the 5 unstable dyads, the red rat was treated with ondansetron (1 mg/kg b.wt, i.p), the other animal was kept untreated, injected with 0.3 ml normal saline solution, i.p. All treatments have been administered half an hour before testing the competition. The competition scores of all rats have been measured in seven daily sessions over a week period.

In the third week of treatment, winners of the first group were treated with fluoxetine HCl (10 mg/kg b.wt., i. p); whereas their corresponding partner losers were treated with ondansetron (1 mg/kg b.wt, i.p). Winners of the second group were treated with Ondansetron (1 mg/kg b.wt, i.p), whereas their corresponding partner losers were treated with fluoxetine

HCl (10 mg/kg b.wt., i.p). In the unstable dyads, the blue rat received fluoxetine HCl (10 mg/kg b.wt., i.p), whereas the red rat received ondansetron (1 mg/kg b.wt, i.p). All treatments have been administered half an hour before testing the competition. The competition scores of all rats have been measured in seven daily sessions over a week period.

**Determination of Serotonin Concentration in Rat Brain**

Rat Serotonin (ST) ELISA Kit was used to determine the concentration of 5-HT in the brains of competing rats. (Cat. No: MBS9362408). A standard curve was constructed by plotting the mean OD for standards on the Y axis against the concentration on the X axis and a best-fit curve was drawn through the points on the graph. These calculations were analyzed with Excel and the best-fit line was determined by regression analysis. Then, the unknown concentrations were extrapolated from the standard curve.

**Statistical analysis**

Average competition scores of winners and losers in each treatment group were expressed as mean ± S.D. Statistical analysis was performed using Microsoft Excel software. Statistical significance for differences between mean scores of treatment groups was tested by one-way ANOVA followed by Bonferroni's post-hoc test. Paired t-test was performed to detect significant difference between competition scores before and after treatment. The confidence limit of $p \leq 0.05$ was considered statistically significant.

**Results**

**Competition Sessions and Rank Assessment of Animals**

Unpaired t-test showed that there was a significant difference between average competition score of winners and average competition score of losers in stable dyads of rats group (1-12); (winners, 51.25 ± 7.29 vs losers, 18.75 ± 7.2, $p \leq 0.001$, Figure **1A**). Moreover, the unpaired t-test shows that there was a significant difference between average competition score of winners and average competition score of losers in stable dyads of rats group (13-20); (winners, 47.71 ± 7.8 vs losers, 22.29 ± 7.83, $p \leq 0.001$, Figure **1B**). In addition to this, unpaired t-test analysis of unstable dyads shows that there was no significant difference between the average competition score of red rats and blue rats (Red, 35.6± 5.03 vs blue, 34.4 ± 5.03, Figure **1C**).

**Treatment of Winners of Stable Dyads and Blue Rats of Unstable Dyads**

**1.1.1. Competition scores after treatment of original winners with Fluoxetine HCl (10 mg/kg b.wt., i.p) in stable dyads of group (1-12)**

ANOVA shows that there was a significant difference between

different groups (F= 30.83, p≤ 0.001). Paired t-test shows that there was a significant difference between the average competition scores of winners before and after treatment (before treatment 50.86 ±7.78 vs after treatment, 31.14 ± 4.15, p≤ 0.001, Figure **2A**), the average competition scores of winners decreased significantly after treatment with Fluoxetine HCl. Consequently, the competition scores of untreated losers increased significantly (losers before FLX-treated winners, 19.14 ± 7.78 vs untreated losers, 38.86 ± 4.15, p≤ 0.001, Figure **2A**). Furthermore, unpaired t-test shows that there was a significant difference between the average competition scores of winners before treatment with Fluoxetine HCl and average competition scores of losers (original score of winners -before treatment 50.86 ±7.78 vs original score of losers, 19.14 ± 7.78, p≤ 0.001, Figure **2A**).

**Competition scores after treatment of original winners with Ondansetron (1 mg/kg b.wt, i.p.) in stable dyads of group (13-20)**

ANOVA shows that there was a significant difference between groups (F= 30.05, p≤ 0.001). Paired t-test shows that there was no significant difference between the average competition scores of winners before and after treatment with Ondansetron (1 mg/kg b.wt, i.p), (before treatment 47.71 ± 7.83 vs after treatment, 44.86 ± 4.71). Also, the competition scores of untreated losers did not change significantly (losers before OND-treated winners, 22.29 ± 7.78 vs untreated losers, 25.14 ± 4.15) see Figure **2B**. Furthermore, unpaired t-test shows that there was a significant difference between the average competition scores of winners after treatment with Ondansetron and untreated losers (after treatment, 44.86 ± 4.71 vs untreated losers, 25.14 ± 4.71, p≤ 0.001, Figure **2B**).

**Treatment of Blue Rats of Unstable Dyads with Fluoxetine HCl (10 mg/kg b.wt., i.p.)**

ANOVA showed that there was a significant difference between groups (F= 5.77, p≤ 0.01). Paired t-test showed that there was no significant difference between the average competition scores of blue rats before and after treatment with Fluoxetine HCl (10 mg/kg b. wt., i.p.), (before treatment 34.4 ± 5.03 vs after treatment, 30.2 ± 1.30). Also, the competition scores of saline-treated red rats did not change significantly (Red before treatment of blues, 35.6 ± 5.03 vs Red untreated 39.8 ± 1.30) see Figure **2C**. Furthermore, unpaired t-test shows that there was no significant difference between the average competition scores of blue and red rats before treatment (blue rats, 34.4 ± 5.03 red rats, 35.6 ± 5.03). In contrast, unpaired t-test shows that there was a significant

difference between blue rats after treatment with Fluoxetine HCl and untreated red rats (Blue rats after treatment, 30.2 ± 1.30 vs untreated red rats 39.8 ± 1.30, p≤ 0.01) see Figure **2C**.

**Treatment of losers of Stable Dyads and Red Rats of Unstable Dyads**

**Competition scores after treatment of original losers with Ondansetron (1 mg/kg b.wt, i.p) in stable dyads of group (1-12)**

ANOVA showed that there was a significant difference between groups (F= 38.59, p≤ 0.001). Paired t-test showed that there was a significant difference between the average competition scores of losers before and after treatment (before treatment 19.14 ± 7.78 vs after treatment, 39.14 ± 2.12, p≤ 0.001, Figure **3A**). Consequently, the competition scores of untreated winners decreased significantly (Winners before treatment of losers 50.86 ±7.78, vs untreated winners 30.86 ± 2.12, p≤ 0.001, Figure **3A**). Furthermore, unpaired t-test shows that there was a significant difference between the average competition scores of losers before treatment with Ondansetron and original winners- before treatment (original score of losers -before treatment 19.14 ±7.78 vs original score of winners, 50.86 ± 7.78, p≤ 0.001, Figure **3A**). It was also shown that there was a significant difference between the score of losers after treatment with Ondansetron (39.14 ± 2.12, p≤ 0.001) and average competition scores of untreated winners (30.86 ± 2.12, p≤ 0.001, Figure **3A**).

**Competition scores after treatment of original losers with Fluoxetine HCl (10 mg/kg b.wt, i.p) in stable dyads of group (13-20)**

ANOVA shows that there was a significant difference between groups (F= 34.15, p≤ 0.001). Unpaired t-test shows that there was a significant difference between the average competition scores of winners and losers before treatment with Fluoxetine HCl (original score of winners, 47.71 ± 7.83 vs original score of losers -before treatment 22.29 ±7.83, p≤ 0.001, Figure **3B**). It was also shown that there was a significant difference between the score of losers after treatment with Fluoxetine HCl (24 ± 4, p≤ 0.001) and average competition scores of untreated winners (46 ± 4.01, p≤ 0.001, Figure **3B**). Furthermore, paired t-test shows that there was no significant difference between the average competition scores of losers before and after treatment (before treatment 22.29 ± 7.83 vs after treatment, 24 ± 4.01, Figure **3B**). The competition scores of untreated winners did not change significantly (Winners before treatment of losers 47.71 ±7.83, vs winners after treatment of losers-untreated 46 ± 4.01, Figure **3B**).

**Treatment of Red Rats of Unstable Dyads with Ondansetron (1 mg/kg b.wt, i.p)**

ANOVA showed that there was a significant difference between groups (F= 7.94, p≤ 0.001). Paired t-test showed that there was a significant difference between the average competition scores of blue rats before treatment of red rats and untreated blue rats, (before treatment 35.6 ±5.03 vs untreated, 29.40 ± 1.14, p≤ 0.05). Also, there was a significant difference between the average competition scores of red rats before and after treatment with Ondansetron (1 mg/kg b. wt., i.p.), (before treatment, 34.4 ± 5.03 vs after treatment 40.6 ± 1.14, p≤ 0.05) see Figure **3C**. Moreover, Unpaired t-test shows that there was no significant difference between the average competition scores of blue and red rats before treatment (blue rats, 35.6 ±5.03 vs red rats, 34.4 ± 5.03). In contrast, unpaired t-test shows that there was a significant difference between red rats after treatment with Ondansetron and untreated blue rats (Red rats after treatment, 40.6 ± 1.14 vs Untreated blue rats 29.4 ± 1.14, p≤ 0.001) see Figure **3C**.

**Simultaneous Treatment of Both Winners and losers of Stable Dyads & Blue and Red Rats of Unstable Dyads.**

**Competition scores after simultaneous treatment of winners with Fluoxetine HCl (10 mg/kg b.wt., i.p.) and losers with Ondansetron (1 mg/kg b.wt., i.p.) in stable dyads of group (1-12)**

ANOVA showed that there was a significant difference between groups (F= 40.86, p≤ 0.001). Unpaired t-test showed that there was a significant difference between average competition score of winners and average competition score of losers before treatment (winners, 50.86 ± 7.78 vs losers, 19.14 ± 7.78, p≤ 0.001, Figure **4A**). Furthermore, unpaired t-test shows that there was a significant difference between average competition score of winners after treatment with fluoxetine and average competition score of losers after treatment with ondansetron (winners, 30 ± 1.63 vs losers, 40 ± 1.63, p≤ 0.001, Figure **4A**). Moreover, paired t-test shows that there was a significant difference between the average competition scores of winners before and after treatment with fluoxetine (before treatment 50.85 ± 7.78 vs after treatment, 30 ± 1.63, p≤ 0.001, Figure **4A**). In addition to this, paired t-test shows that there was a significant difference between the average competition scores of losers before and after treatment with ondansetron (before treatment 19.14 ± 7.78 vs after treatment, 40 ± 1.63, p≤ 0.001, Figure **4A**).

**Competition scores after simultaneous treatment of winners with Ondansetron (1 mg/kg b.wt., i.p.) and losers with**

**Fluoxetine HCl (10 mg/kg b.wt., i.p.) in dyads group (13-20)**

ANOVA showed that there was a significant difference between groups (F= 29.27, p≤ 0.001). Unpaired t-test showed that there was a significant difference between average competition score of winners and average competition score of losers before treatment (winners, 47.71 ± 7.83 vs losers, 22.29 ± 7.83, p≤ 0.001, Figure **4B**). Furthermore, unpaired t-test shows that there was a significant difference between average competition score of winners after treatment with ondansetron and average competition score of losers after treatment with Fluoxetine HCl (winners, 41.29 ± 1.70 vs losers, 28.71± 1.70, p≤ 0.001, Figure **4B**). Moreover, paired t-test shows that there was no significant difference between average competition score of winners before and after treatment with ondansetron (winners before, 47.71 ± 7.83 vs winners after, 41.29± 1.70, Figure **4B**). In addition to this, paired t-test shows that there was no significant difference between average competition score of losers before and after treatment with Fluoxetine HCl (losers before, 22.29 ± 7.83 vs losers after, 28.71 ± 1.70, Figure **4B**).

**Competition scores after simultaneous treatment of Blue Rats with Fluoxetine HCl (10 mg/kg b.wt., i.p.) and Red Rats with Ondansetron (1 mg/kg b.wt., i.p.) in Unstable dyads**

ANOVA showed that there was a significant difference between groups (F= 15.31 p≤ 0.001). Unpaired t-test showed that there was a significant difference between the average competition scores of blue rats after treatment with Fluoxetine HCl and red rats after treatment with Ondansetron (after Fluoxetine HCl, 25.8 ± 3.42 vs after Ondansetron, 44.2 ± 3.42, p≤0.001, Figure **4C**). Furthermore, unpaired t-test shows that there was no significant difference between the average competition scores of blue rats before treatment with Fluoxetine HCl and red rats before treatment with Ondansetron (before Fluoxetine, 35.6 ± 5.03 vs before Ondansetron, 34.4 ± 5.03, Figure **4C**). Moreover, paired t-test shows that there was a significant difference between the average competition scores of blue rats before and after treatment with Fluoxetine HCl (before treatment 35.6 ± 5.03 vs after treatment, 25.8 ± 3.42, p≤0.05, Figure **4C**). In addition to this, paired t-test shows that there was a significant difference between the average competition scores of red rats before and after treatment with Ondansetron (before treatment 34.4 ± 5.03 vs after treatment, 44.2 ± 3.42, p≤0.05, Figure **4C**).

**Effect of Treatment with Either Ondansetron or Fluoxetine on Brain levels of Serotonin (ng/g**

**tissue) in Winners, losers, and Unstable Rats**

Ondansetron has shown a depressive effect on the serotonin levels in the brains of all OND-treated rats. In contrast, serotonin levels have elevated in the brains of all FLX-treated rats, as compared to the serotonin level in brain of both OND-treated rats and untreated control rats (Figure **5A** and **5B**). Furthermore, unpaired t-test has shown a significant difference between serotonin level in brains of all Fluoxetine-treated rats and Ondansetron-treated rats (Fluoxetine, 38.58 ± 4.80 ng/g tissue vs Ondansetron, 18.8 ± 3.82 ng/g tissue, $p \leq 0.001$) Figure **5B**.

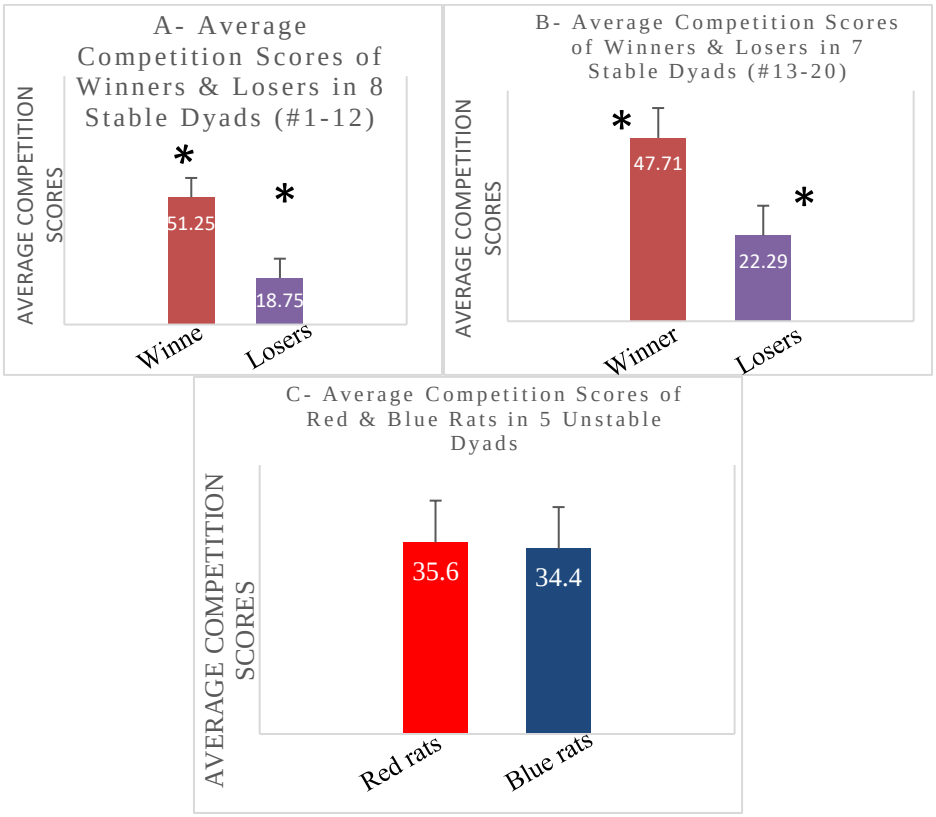


**Figure 1.** Average competition scores of winners and losers in 8 stable dyads (#1-12) **(A).** Average competition scores of winners and losers in 7 stable dyads (#13-20) **(B).** Average competition scores of red & blue rats in 5 unstable dyads **(C).** Variables are described as mean ± S.D. The asterisk symbol (*) above the bars means that there is a significant difference between different groups at $p \leq 0.001$.

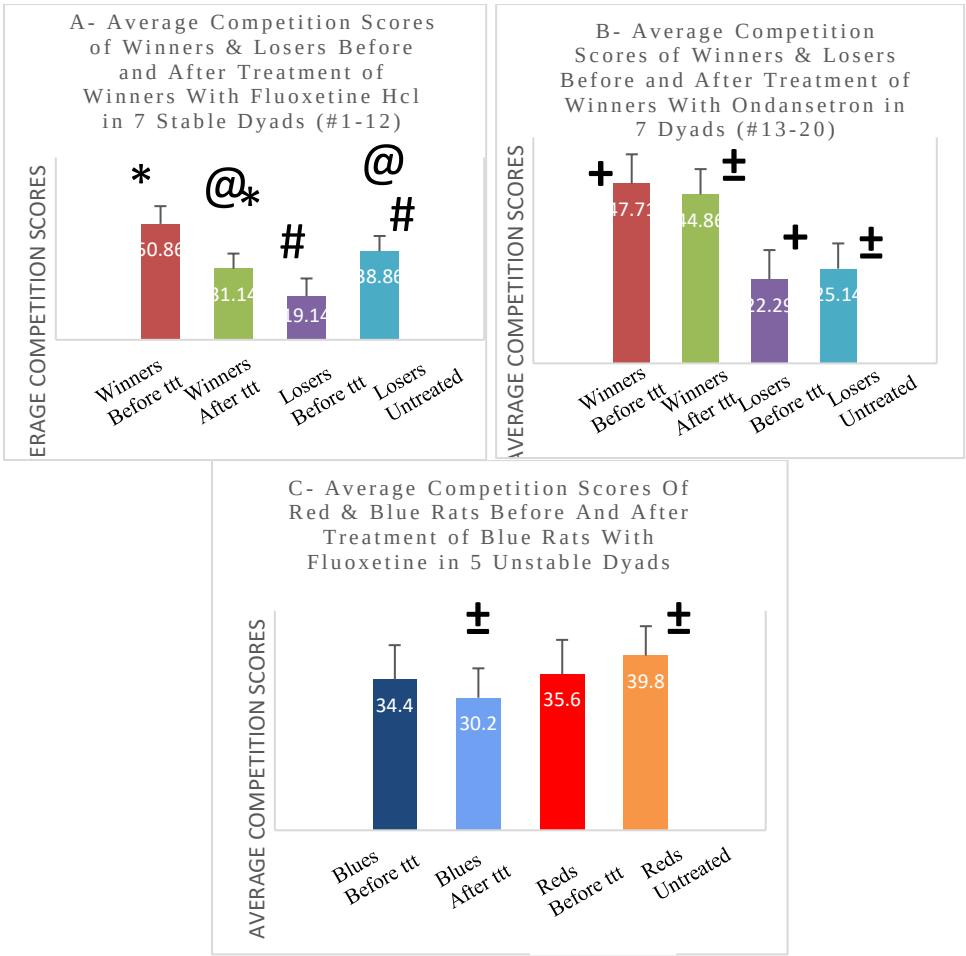


**Figure 2.** Average competition scores of winners & losers before and after treatment of winners with Fluoxetine HCl (10 mg/kg b. wt., i.p.) in 7 stable dyads (#1-12) **(A).** Average competition scores of winners & losers before and after treatment of winners with ondansetron (1 mg/kg b. wt., i.p.) in 7 dyads (#13-20) **(B).** Average competition scores of red & blue rats before and after treatment of blue rats with fluoxetine HCl (10 mg/kg b. wt., i.p.) in 5 unstable dyads **(C).** Variables are described as mean ± S.D. and evaluated using one-way ANOVA followed by Bonferroni's post hoc test. Different symbols (*, #) above the bars mean that paired t-test shows a significant difference between groups at $p \leq 0.001$, while symbols (+, ±) above the bars mean that unpaired t-test shows a significant difference between groups at $p \leq 0.01$.

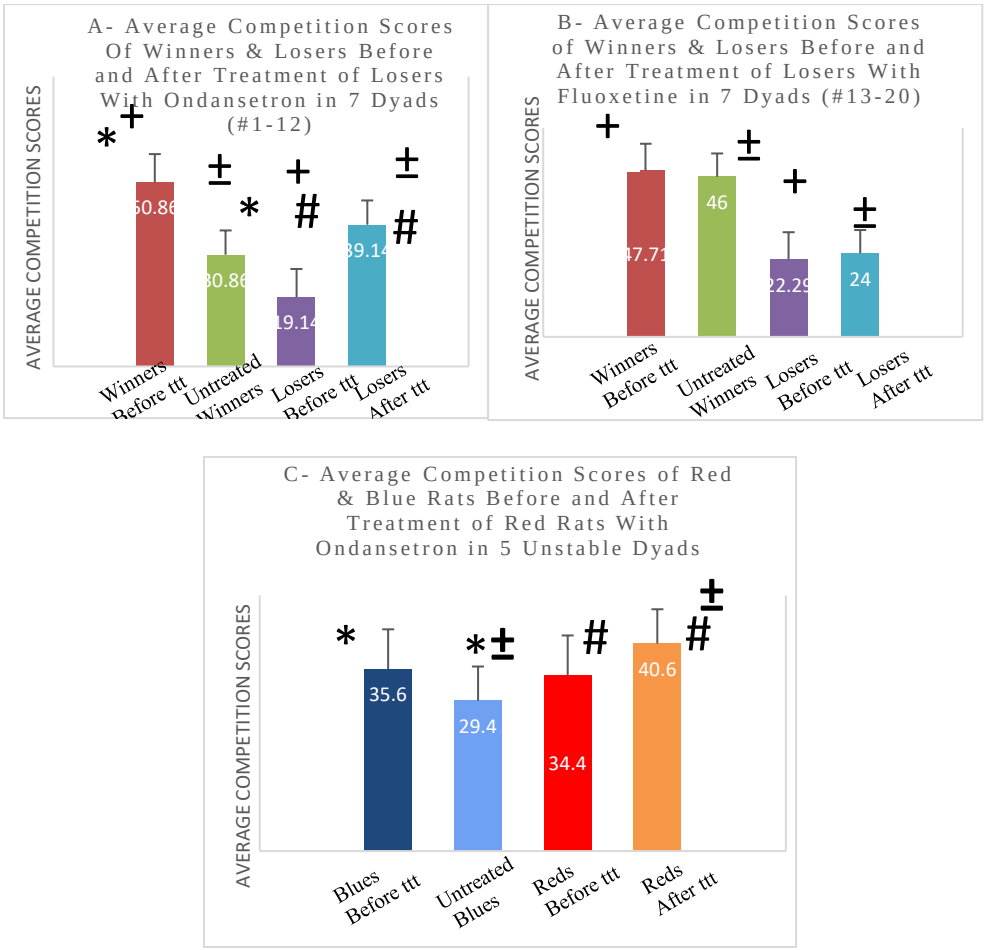


**Figure 3.** Average competition scores of winners & losers before and after treatment of losers with ondansetron in 7 dyads (#1-12) **(A).** Average competition scores of winners & losers before and after treatment of losers with Fluoxetine HCl in 7 dyads (#13-20) **(B).** Average competition scores of red & blue rats before and after treatment of red rats with ondansetron in 5 unstable dyads **(C).** Variables are described as mean ± S.D. and evaluated using one-way ANOVA followed by Bonferroni's post hoc test. Different symbols (*, #) above the bars mean that paired t-test shows a significant difference between groups at $p \leq 0.05$, while symbols (+, ±) above the bars mean that unpaired t-test shows a significant difference between groups at $p \leq 0.001$.

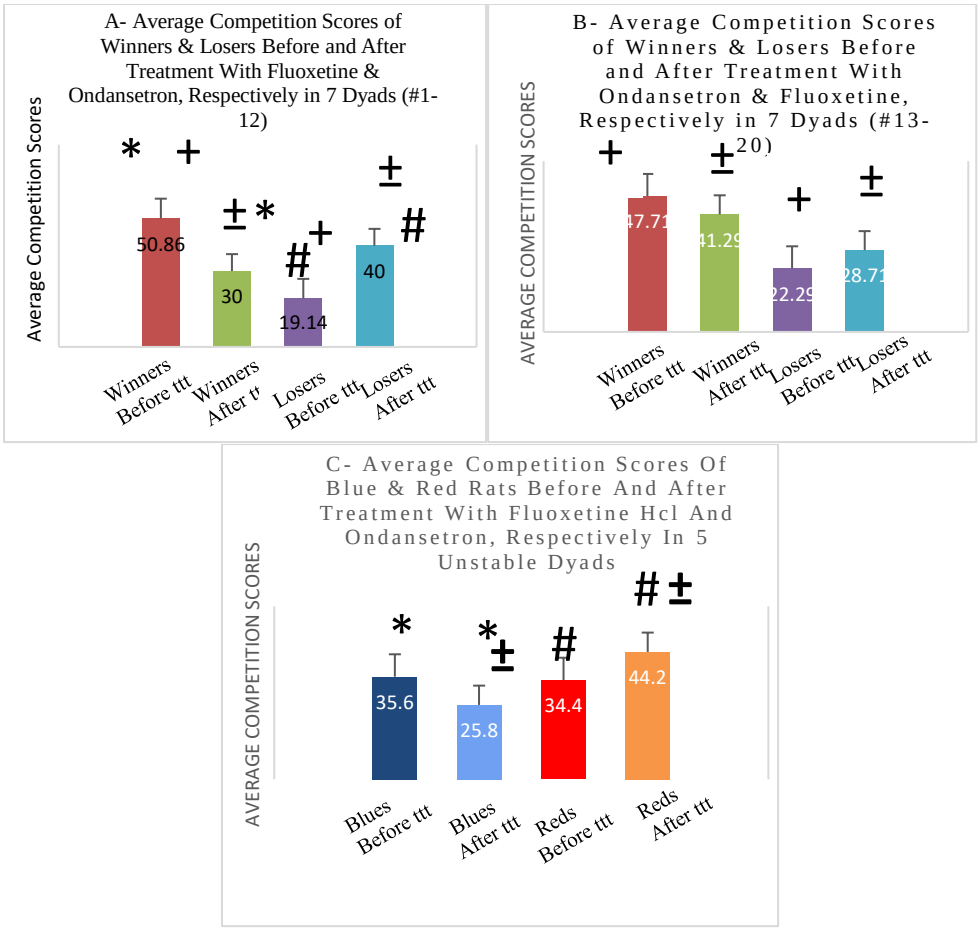


**Figure 4.** Average competition scores of winners and losers before and after treatment with Ondansetron and Fluoxetine HCl, respectively in 7 stable dyads (#13-20) **(A).** Average competition scores of winners & losers before and after treatment with ondansetron & fluoxetine, respectively in 7 Dyads (#13-20) **(B).** Average competition scores of blue & red rats before and after treatment with Fluoxetine HCl and Ondansetron, respectively in 5 unstable dyads **(C).** Variables are described as mean ± S.D. and evaluated using one-way ANOVA followed by Bonferroni's post hoc test. Different symbols (*, #) above the bars mean that paired t-test shows a significant difference between groups at $p \leq 0.05$, while symbols (+, ±) above the bars mean that unpaired t-test shows a significant difference between groups at $p \leq 0.001$.

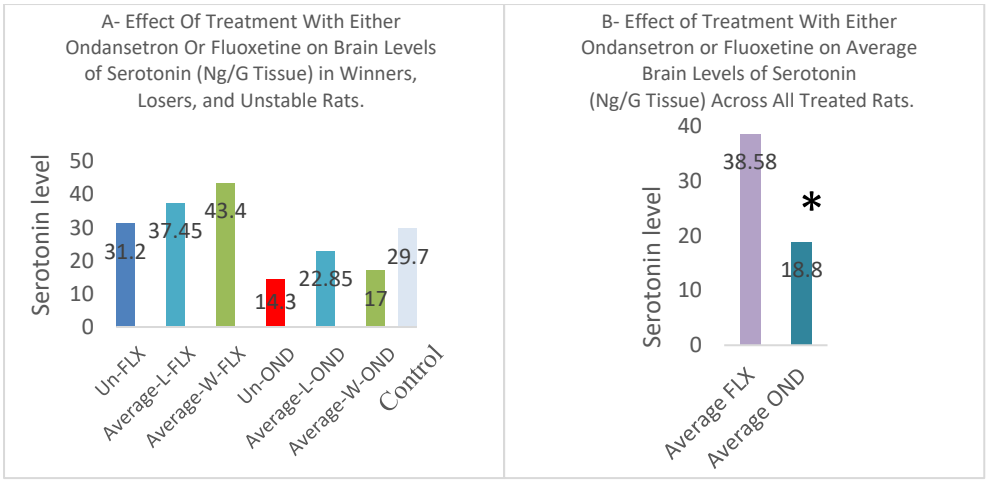


**Figure 5.** Effect of treatment with either Ondansetron or Fluoxetine HCl on rats' brain level of serotonin in winners, losers, and unstable rats **(A).** Effect of treatment with either Ondansetron or Fluoxetine HCl on average brain levels of serotonin (ng/g tissue) across all treated rats **(B).** Variables are described as mean ± S.D. The asterisk symbol (*) above the bars means that there is a significant difference between different groups at $p \leq 0.001$.

**Discussion**

In the present animal model for food competition, home cage partners competed for the consumption of sucrose pellets. It is crucial to note that competition sessions were performed in the home cage, thus decreasing the effect of the experimenter in the competition process; further, running the competition sessions in the home cages of animals prevented anxiety or exploratory behaviors, which is shown by animals in new environments. In addition, food delivery was performed in an intermittent repeated manner, which enhanced competition. Indeed, the outcome of the competition for sucrose pellets is certainly affected by the fights and other events in the home cage proceeded the competition session; in other words, dominance rank might have been established even before the start of the competition session ***(Cummins, 2021)***. Further, in the current model of food competition, rats were individually trained on the consumption of sucrose pellets in a specified cage for training and immediately returned to their home cage; that is to avoid the interrupting effect of the partner of home cage; this also avoided the inhibitory effect of the partner on the other rat's feeding behavior ***(Wang et al., 2014)***. In summary, here we provide a new model of competition in the home cage of food-deprived rats. In the present model, for rank stability

evaluation, rank stability was evaluated over 7 days' time period. It has been shown in a previous study that mice have maintained the same rank position in 59% of the dyads ***(Wang et al., 2011)***. In comparison, our results demonstrated stability of rank order in fourteen out of twenty dyads (70% stability).

Competition has always been associated with aggression; aggression has been considered as a competitive tool to win the competition ***(Costa et al., 2021)***. Since female rats exhibit lower level of aggression ***(Lozano-Montes et al., 2019)***, male albino rats, which show higher aggression level and thereby higher competitiveness were used in the present study.

It is well established that central 5-HT activity has an inhibitory effect on aggression in animals and human. This relationship was proved by assessing the level of 5-HT separated from the CSF ***(Okaty et al., 2019)***. Increased levels of aggression have been linked to low serotonin activity in the brain of aggressive mice ***(Leclair et al., 2021)*** Further, decreased levels of 5-hydroxyindol acetic acid (5-HIAA) in cerebrospinal fluid (CSF) are found in violent humans ***(Seo et al., 2008)*** and aggressive macaques ***(Sapolsky, 2004).*** Our results confirmed previous findings, where low activity of 5-HT was detected in the brains of OND-treated winners, whereas FLX-treated losers demonstrated high concentration of 5-HT. Therefore, the present results showed that fluoxetine-treated rats exhibited a decline in the competitive behavior, which was accompanied by increased 5-HT concentrations in their brains, and vice versa, in rats treated with ondansetron. Reduced aggression was observed in subjects treated with fluoxetine. This effect of fluoxetine was evident in the present study, since fluoxetine-treated rats showed elevated levels of 5-HT and decreased competition scores.

Some authors ***(Costall et al., 1989; Pistovcakova et al., 2011)*** reported an anxiolytic effect of ondansetron in rats. This effect may represent an additive effect of ondansetron on competitive behavior of rats. The anxiolytic properties of the drug may have enhanced the competitive behavior and improved competitive performance of rats, in the present study, to become winners. In addition, it has been shown that acute ondansetron treatment showed antidepressant-like effect ***(Ramamoorthy et al., 2008)***, which is another possible effect of ondansetron, in the present study, that may have contributed to the improved competitive behavior of ondansetron-treated rats.

One limitation of our work was the use of a specific population for our study. Although we did not anticipate major problems in using the food competition test in other rat strains, behavioral differences

have been already reported in several social and nonsocial behaviors between long-Evans, Wistar and Sprague–Dawley rats ***(Netser et al., 2020)***. It could be possible that different rat strains would react differently to the subtle conflict we induce in the cage during the food competition test, and that acute aggressive encounters could be observed depending on the strain.

## Conclusion

It was evident that 5-HT agonist, fluoxetine, and 5-HT antagonist, ondansetron, had significant decreasing and increasing effects, respectively, on the competitive behavior of rats; further, FLX and OND showed enhancing and depressive effects, respectively, on 5-HT concentrations in the brains of the currently competing rats. It is recommended to further investigate the therapeutic benefits of fluoxetine and ondansetron in treating personality disorders including impulsive aggression in certain patients.

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# التأثيرات المثبطة والمحفزة لكل من محفز السيروتونين، فلوكستين، ومضاد السيروتونين، أوندانسيترون، علي التوالي، على تنافس الجرذان للطعام.

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## الملخص العربى

**الهدف من البحث والخلفية العلمية:** أثبتت الدراسات السابقة أن زيادة العدوانية فى الحيوانات يتزامن مع نقص نسبة السيروتونين فى مخ هذه الحيوانات، وأن زيادة تركيز السيروتونين فى مخ هذه الحيوانات يرتبط بنقص مستوى العدوانية فى سلوكها. تهدف الدراسة الحالية الى بحث تأثير الفلوكستين، المنشط للسيروتونين فى مخ الجرذان الذكور البضاء وكذلك تأثير الأوندانسيترون، المثبط لنشاط السيروتونين فى مخ الجرذان، على السلوك التنافسى فى الجرذان وكذلك تأثير هذه الأدوية على مستوى السيروتونين فى مخ هذه الجرذان المتنافسة.

**طرق البحث:** أربعون جرذا أبيضا من الذكور تم تقسيمهم الى عشرين زوجا فى أقفاص منفصلة، تم حرمان جميع الحيوانات من الطعام لمدة ٢٣ ساعة يوميا حتى نهاية الدراسة، أجريت المنافسة على إلتهام أقراص السكر المقدمة يوميا عن طريق قمع زجاجى فى قفصهم الطبيعى لمدة ٧ أيام، الحيوان الذى ينجح فى التهام العدد الأعظم من أقراص السكر على مدى ٥ منافسات يومية يعتبر فائزا والأخر خاسرا، وبالتالى تم تقسيم جميع أزواج الحيوانات الى مجموعة أزواج مستقرة الترتيب الإجتماعى (١٤ زوجا قسمت الى مجموعتين، كل مجموعة قسمت الى فائزين وخاسرين) وأخرى غير مستقرة الترتيب (٥ أزواج). تم علاج الجرذان الفائزة والخاسرة فى جميع المجموعات بالتبادل بالفلوكستين (١٠ ملج/كجم من وزن الجسم) والأوندانسيترون (١ ملج/كجم من وزن الجسم) بالحقن فى تجويف البطن، ودراسة تأثير العلاج بهذه الأدوية على السلوك التنافسى فى هذه الجرذان، وكذلك دراسة تأثير هذه الأدوية على تركيز السيروتونين فى هذه الجرذان المتنافسة.

**النتائج:** لوحظ هبوط فى السلوك التنافسى للحيوانات المعالجة بالفلوكستين، فى حين إستطاع العلاج بالإوندانسيترون أن يرفع من مستوى السلوك التنافسى فى الجرذان المعالجة، إرتبط تأثير هذه الأدوية على السلوك التنافسى للجرذان بالتأثير على تركيز السيروتونين فى مخ هذه الجرذان المتنافسة، حيث لوحظ إرتفاع فى تركيز السيروتونين فى مخ الجرذان المعالجة بالفلوكستين، فى حين إستطاع العلاج باللأوندانسيترون ان يثبط من نشاط السيروتونين فى مخ الجرذان المعالجة،

**الإستنتاج والتوصيات:** إستطاع العلاج بالفلوكستين أن يثبط من مستوى السلوك التنافسى للجرذان، فى حين لوحظ إرتفاعا فى مستوى السلوك التنافسى فى الجرذان بعد العلاج بالأوندانسيترون، وكان للعلاج بهذه الأدوية تأثرات عكسية على تركيز السيروتونين فى مخ هذه الجرذان المعالجة. توصيات هذه الدراسة تشمل إجراء المزيد من الأبحاث الإكلينيكية لكشف تأثير الفلوكستين فى علاج المرضى العدوانيين وكذلك الكلاب الخطيرة.

**الكلمات المفتاحية:** أوندانسيترون، فلوكستين، الهرم الإجتماعى، السيروتونين