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Research

Effects of serotonin reuptake inhibition and environmental enrichment on aggression in Betta fish (*Betta splendens*)

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Many species exhibit aggressive behaviors, and the intensity of aggression can be modulated by different factors. For instance, the neurotransmitter serotonin is negatively correlated with aggression, and selective serotonin reuptake inhibitors (SSRIs) are drugs used to increase the extracellular serotonin levels by blocking the reabsorption of serotonin into presynaptic neurons, leading to decreased aggression. Also, environmental enrichment—when an animal is placed in an artificial habitat that tries to mimic its natural habitat—has been shown to decrease aggression in captive animals. However, research on the interaction of factors that regulate aggression are not completely known and, specifically, the relationship between serotonin and environmental enrichment is not completely understood. The purpose of the experiment was to determine if environmental enrichment and SSRIs will decrease aggressive behaviors more so than each factor alone. It was hypothesized that male Betta fish (*Betta splendens*) exposed to SSRIs and held in enriched environments will show lower levels of aggression compared with controls, SSRIs alone, or enriched environments alone. The results revealed that SSRIs decrease aggressive behaviors, but environmental enrichment was not a significant predictor of aggressive behaviors. Likewise, the interaction between the SSRIs and environmental enrichment was not a significant predictor of aggressive behaviors. Therefore, our study supports previous studies that found SSRIs decrease aggression but also suggests that environmental enrichment either does not affect aggression in Betta fish or that our operationalization of environmental enrichment was not sufficient to change aggressive behaviors.

AUTHOR SUMMARY

Aggression has long been studied and there are many different circumstances that can provoke aggressive behaviors in different species. Serotonin and the level of enrichment of the environment are two variables that can affect an individual's level of aggression. The study examined how Betta fish aggression was impacted when exposed to a serotonin reuptake inhibitor and an enriched environment. The study did not find a significant change in aggression when Betta fish were exposed to environmental enrichment alone or environmental enrichment and SSRIs simultaneously. However, SSRIs alone did decrease aggressive behaviors. Therefore, this study did not find that SSRIs and environmental enrichment interacted to modulate aggression in Betta fish.

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INTRODUCTION

Aggression is a behavior that has been studied in many different species. From the neurological processes associated with it to its environmental triggers, aggression is a complex behavior. Aggressive behaviors can be triggered when organisms defend territories, are looking for mates, food, or other resources that are necessary for survival (Maher and Lott, 2000). Without these aggressive behaviors, animals could be excluded from essential resources, thus lowering their chances of reproduction and survival.

When trying to understand aggression, researchers have gained much information by examining the physiological mechanisms that produce aggressive behaviors. One way to examine this is to study the neural and hormonal mechanisms of aggression. For example, low levels of serotonin, an amine neurotransmitter, have been linked to higher levels of aggression (Summers et al., 2005). Further, factors that modulate the levels of serotonin have also been correlated with changes in aggression in some species (Ferrari et al., 2005). Selective serotonin reuptake inhibitors (SSRIs) are widely used to treat conditions including depression and anxiety (Lacasse and Leo, 2005), and they operate by allowing serotonin to exert its effects for a longer duration. In addition to treatment for anxiety and depression, SSRIs have also been shown to decrease aggression via the same mechanism. For example, an SSRI administered to Betta fish (*Betta splendens*) resulted in decreased boldness and decreased frequency and duration of aggressiveness (Dziewecynski et al., 2016). The anti-aggressive effects were attributed to the SSRI's sedative effect that increased serotonin levels and decreased arousal to external stimuli. In another study, an SSRI decreased paternal territorial aggressive behaviors after spawning and hatching of the larvae (Forsatkar et al., 2014). These studies demonstrate that SSRI exposure can decrease levels of aggression via the physiological mechanism of increasing serotonin levels.

Along with neural modulators of aggression, there are environmental factors that can affect aggressive behaviors that are produced when organisms are competing for necessary resources for survival and reproduction such as food, mates, and dominance. As such, variation in these environmental factors can impact an individual's aggression, and an individual's history with the environment can produce differential levels of aggression depending on the context (Robinson et al., 2019). For example, if an individual was constantly exposed to a highly competitive environment, with low accessibility to food and shelter, their aggressive behaviors could be greater, and initiated quicker, than an individual who was consistently exposed to an environment with an abundance of food and less competition for shelter (Ashley, 2007). Thus, the environment an organism has been exposed to can organize and impact aggressive behaviors in subsequent contexts.

One important environmental factor that can affect an individual's development and behavior, particularly in captivity, is environmental enrichment. Environmental enrichment is when an organism is surrounded by a stimulating environment that attempts to more closely reflect the complexity of the natural environment. This can be achieved in different ways such as including more plants, structures, or conspecifics. In contrast, a non-enriched environment would be devoid of such structures or conspecifics. Similarly, wild environments are more enriched than the laboratory environment because of the increased space, plants, and other conspecifics. The psychological and physiological health of captive animals are thought to be improved with environmental enrichment as they are exposed to a similar variety of environmental factors that can be found in the wild (Robinson et al., 2019). Stress and anxiety behaviors associated with captive environments can be mitigated with environmental enrichment. Previous studies have shown that individuals in enriched captive environments demonstrate increased survival when released into the wild, improved learning ability, increased behavioral flexibility, and increased neural plasticity compared with individuals reared in non-enriched captive environments (Brydges and Braithwaite, 2009; Collymore et al., 2015; Johnsson et al., 2014; Rosengren et al., 2017). Environmental enrichment has also been shown to affect aggressive behaviors of individuals housed in enriched environments, reflected by a decrease in agonistic behaviors (Giles et al., 2018). In a study of Atlantic salmon (Salmo salar), a lower density of salmon and the availability of shelter for retreat from stressful social interactions decreased conspecific aggression, which then positively affected growth and development (Rosengren et al., 2017). Thus, enrichment of the captive environment can modulate development and behavior, including modulating aggressive behaviors.

While SSRIs and environmental enrichment can both decrease aggression alone, we still do not fully understand how SSRIs and environmental enrichment interact with each other to produce variation in aggressive behaviors. In this study, we were interested in how fluoxetine, an SSRI, and environmental enrichment affect aggressive behaviors in Betta fish, a territorial fish that demonstrates high levels of male-male aggression. It is predicted that male Betta fish exposed to SSRIs and held in enriched environments will show lower levels of aggression. Specifically, both the environmental enrichment treatment and the SSRI treatment will lead to a decrease in aggressive behaviors, including a shorter amount of time in frontal and broadside display, fewer 90° turns, and a longer latency to display compared to the control conditions. Further, it is predicted that the interaction between SSRI and environmental enrichment will decrease aggressive behaviors to an even greater extent when the Betta fish are placed in aggression provoking scenarios.

METHODS

Subjects and materials

Twenty-four male Betta fish were bought from a commercial vendor. The exact ages of the fish were unknown, but body length indicated they were all adults (Pleeging and Moons, 2017). The fish were immediately housed and randomly assigned to environmental enrichment treatments. Half of the fish were housed in an enriched environment (two plastic plants and gravel substrate), and the other half were housed in a simple environment (no plants or gravel, just water). The fish were kept in their respective treatment groups for two weeks. All tanks had a tapered design with the top measuring 21 cm x 12 cm and the bottom measuring 18.5 cm x 10.5 cm, and a depth of 14 cm. The fish were on a light/dark cycle of 10 hours light and 14 hours dark. The fish were fed 6-8 pellets of Betta fish food every day and their water was changed weekly.

Testing methodology

For the fluoxetine treatment, we followed a modified protocol of Lynn et al. (2007). All fish were transferred to cylindrical 400 mL capacity containers the evening before testing and half were given a single dose of fluoxetine HCl (6.0 μg/mL), which has shown to be sufficient to modulate aggressive behaviors in Betta fish (Lynn et al., 2007). The next morning, subjects were placed in 30 cm x 20 cm x 15 cm aquariums with only water and tested within 3 hours of lights on approximately 16 hours after the administration of fluoxetine. The aquarium was enclosed on three sides with neutral brown construction paper and had a lid. A 10 cm mark from the short edge of the tank was placed to indicate where the mirror would be placed. To elicit aggressive behaviors, a mirror (11.5cm x 15cm) was placed in the tank and flushed along the short end. The fish was given 5 minutes to express an aggressive behavior (see behaviors below) and if the fish did not express an aggressive behavior in that time period, the fish was coaxed within the 10 cm marker gently with a ruler to ensure it likely had visual contact with the mirror. Once the first aggressive behavior was recorded or 5 minutes elapsed, the fish was monitored for an additional 5 minutes. After the final 5 minutes, the fish was returned to its home enclosure for recovery. Each Betta fish was tested twice with a counterbalanced design. The fish were either given fluoxetine or no fluoxetine (control) for the first day of testing. Then, three days later, the control and fluoxetine experimental groups were switched and tested again. Therefore, the fish given fluoxetine the first day of testing served as its own control the second day of testing, and the fish that were not given fluoxetine the first day were treated with fluoxetine on the second day of testing. Half of all the fish that were dosed with fluoxetine were in the enrichment environment group while the other half were in the simple environment; fish were only tested in one type of environment group. Immediately after the second day of testing, all subjects were humanely sacrificed. All trials were recorded so behaviors could be scored. The four aggressive behaviors recorded were: time spent in broadside display, time spent in frontal display, the number of 90 degree turns, and the time latency to first expression of an aggressive behavior. Two researchers blind to the treatment groups analyzed the videos separately to ensure consistent assessment of behaviors; interobserver reliability was evaluated and confirmed. During the second day of testing, one subject died immediately after testing. Since Betta fish are relatively resilient fish, this data point was taken out of the final analysis. Three other fish died between the first and second days and thus, the data for the second day of testing was not included in the analysis. All procedures were approved by the Pennsylvania State University's Institutional Animal Care and Use Committee (protocol #201546456).

Statistical Tests

Differences in aggressive behaviors across treatments were assessed with a repeated- measures general linear model (GLM) for each of the four dependent variables and all analyses were conducted with SPSS for Windows, v. 27 (IBM Corp., Armonk, NY, U.S.A.). Results were considered significant when $p \leq .05$.

RESULTS

Data for averages $\pm$ standard error (SE) of each aggressive behavior are in Table 1. For the latency and broadside displays, the data were square-root transformed to conform to the assumption of equality of variances for the statistical tests.

Table 1. Latency to first aggressive behavior, time spent in frontal and broadside displays, and number of 90° turns in Betta fish who were control or dosed with fluoxetine and housed in either simple or enriched environments. All values are averages $\pm$ SE.

	Treatment Groups			
	Control Enriched	Control Sample	Dosed Enriched	Dosed Sample
Dependent Variables				
Latency to approach (s)	140.6 ± 34.78	23.03 ± 8.34	32.13 ± 12.91	12.3 ± 6.05
Frontal displays (s)	152.54 ± 36.97	38.94 ± 16.86	39.75 ± 17.82	25.0 ± 9.30
Broadside displays (s)	258.03 ± 22.11	7.07 ± 7.07	41.08 ± 29.54	5.13 ± 3.66
Number of 90° turns	235.64 ± 33.43	3.53 ± 2.25	3.11 ± 1.74	2.64 ± 1.37

The overall model indicated that the fluoxetine treatment increased latency ($F_{1,14} = 17.527$, $p = .001$), while environment and the interaction between environment and fluoxetine treatment were not significant predictors of latency ($p = .696$, $p = .729$, respectively) (Table 2, Figure 1).

The overall model indicated that fluoxetine treatment decreased frontal displays ($F_{1,14} = 4.744$, $p = .047$), while environment and the interaction between environment and fluoxetine treatment were not significant predictors of frontal displays ($p = .524$, $p = .905$, respectively) (Table 2, Figure 2).

The overall model indicated that fluoxetine treatment decreased broadside displays ($F_{1,14} = 7.994$, $p = .013$), while environment and the interaction between environment and fluoxetine treatment were not significant predictors of broadside displays ($p = .890$, $p = .897$, respectively) (Table 2, Figure 3).

The overall model indicated that fluoxetine treatment decreased the number of 90° turns ($F_{1,14} = 5.955$, $p = .029$), while environment and the interaction between environment

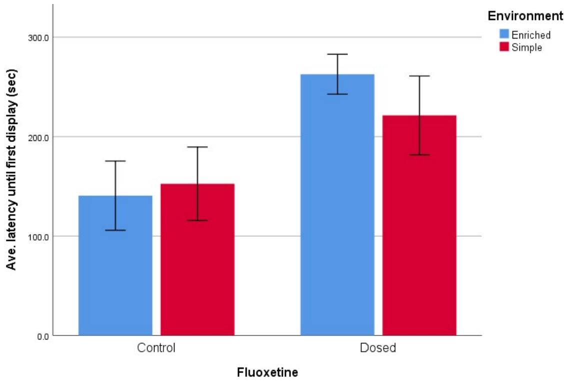

Figure 1. The average latency of first aggressive display (± SE) among the control and fluoxetine-treated betta fish in simple and enriched environments. Fluoxetine-treated individuals demonstrated a greater latency to first aggressive display compared with control individuals (p = 0.001) while there was no significant effect of environment type or the interaction between fluoxetine treatment and environment type (p = 0.524, p = 0.905, respectively).

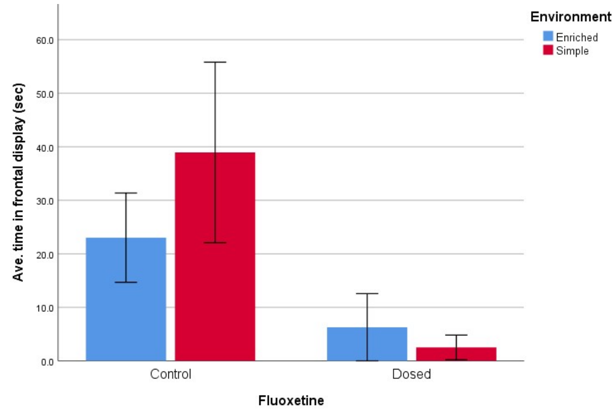

Figure 2. The average time in frontal display (± SE) among the control and fluoxetine-treated betta fish in simple and enriched environments. Fluoxetine-treated individuals spent less time in frontal display when compared with control individuals (p = 0.047) while there was no significant effect of environment type or the interaction between fluoxetine treatment and environment type (p = 0.524, p = 0.905, respectively).

Table 2. p-values for differences in treatment from fluoxetine (control vs. dosed), environmental enrichment (simple vs. complex), and the interaction between fluoxetine and environment treatments. *indicates statistically significant differences at $p \leq 0.05$.

	Independent Variables		
	Fluoxe-tine	Environ-ment	Interac-tion
Dependent Variables			
Latency to approach (s)	0.001*	0.696	0.729
Frontal displays (s)	0.047*	0.524	0.905
Broadside displays (s)	0.013*	0.890	0.897
Number of 90° turns	0.029*	0.554	0.874

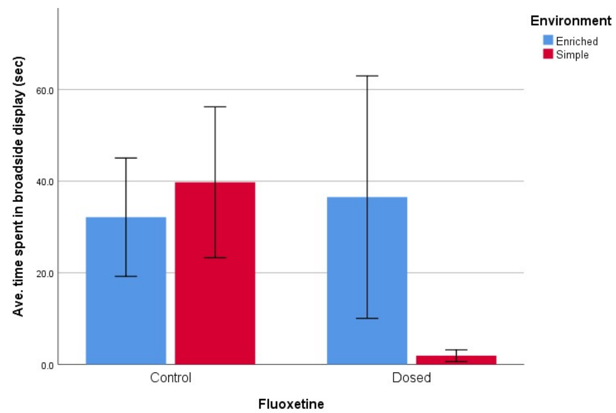

Figure 3. The average time in broadside display (± SE) among the control and fluoxetine-treated betta fish in simple and enriched environments. Fluoxetine-treated individuals spent less time in broadside display when compared with control individuals (p = 0.013) while there was no significant effect of environment type or the interaction between fluoxetine treatment and environment type (p = 0.890, p = 0.897, respectively).

and fluoxetine treatment were not significant predictors of the number of 90° turns (p = .554, p = .874, respectively) (Table 2, Figure 4).

DISCUSSION

Betta fish exposed to the SSRI fluoxetine decreased their aggressive behaviors compared to when they were not exposed to fluoxetine. When exposed to fluoxetine,the fish spent a significantly shorter amount of time in frontal and broadside displays, performed fewer 90° turns, and had a longer latency until the first aggressive display compared to when the fish were not exposed to fluoxetine. However, the results did not show a significant difference between the aggressive behaviors of the environmentally enriched group and the simple environment control group. Unlike the SSRI group, the fish in the environmental enrichment group did not differ in the four aggressive behaviors measured when compared to the control group of fish not exposed to enriched environments. Furthermore, there were no significant effects of the interaction between an enriched environment and fluoxetine. Taken together, we reject the hypothesis that Betta fish exposed to SSRIs and held in enriched environments would show lower levels of aggression. The interaction between SSRIs and environmental enrichment and their impact on aggression was also not significant, thus

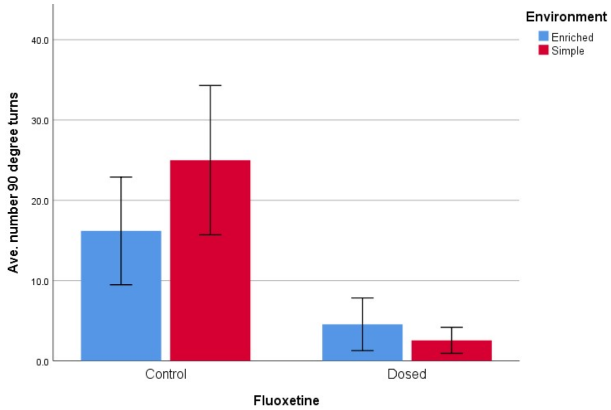

Figure 4. The number of 90° turns (± SE) among the control and fluoxetine-treated betta fish in simple and enriched environments. Fluoxetine-treated individuals had fewer 90° turns when compared with control individuals (p = 0.029) while there was no significant effect of environment type or the interaction between fluoxetine treatment and environment type (p = 0.554, p = 0.874, respectively)

causing the hypothesis to be rejected.

Serotonin is considered one of the most important modulators of aggression (Popova, 2006), particularly in male-male interactions, and many studies have found a negative correlation between serotonin levels and aggressive behaviors in a wide range of species (Summers et al., 2005). Further, reduced activity in serotonin has been linked with clinical depression, suggesting that serotonin may also be involved in the pathophysiology of depression (Cowen and Browning, 2015). As such, the use of SSRIs to increase serotonin levels in the brain and suppress depressive mood is very popular in the United States (Lacasse and Leo, 2005). In the present study, we leveraged the SSRI pharmaceutical to modulate serotonin levels in the brain in order to see the effect on aggressive behaviors. Compared to past research, the results of this study are somewhat contradictory. In regard to the SSRI treatment, the relationship between fluoxetine and aggression levels was similar to that found in past research. Results and past research show that SSRIs have been linked to lower levels of aggression, and Betta fish have shown decreased duration and frequency of aggressive behaviors when exposed to SSRIs (Eisenreich and Szalda-Petree, 2015; Lynn et al., 2007). Another study looked at how fluoxetine impacted both aggression and courtship behaviors in male fish when they were exposed to the drug and found a similar effect of decreased aggression when dosed with fluoxetine; courtship behaviors, however, were unaffected (Dzieweczynski and Herbert, 2012). Mechanistically, the conclusion was that, when fluoxetine is administered it prevents reuptake of the serotonin in the synapse and increases the amount of serotonin available to the organism, leading to a reduction in aggressive behaviors.

While the effects of SSRI were statistically significant, the results of this study were not as clear compared to that of previous studies concerning environmental enrichment and aggression. The present study did not find an effect of environmental enrichment on aggressive behaviors in Betta fish. However, in a study involving black rockfish (Sebastes melanopes), environmental enrichment significantly decreased aggression levels in the fish, as well as lowered levels of basal cortisol, a stress hormone (Zhang et al., 2020). Therefore, when the black rockfish were exposed to environmentally enriched environments, not only did they display lower levels of aggression, but also lower levels of cortisol, indicating lower levels of stress. With lower levels of the stress hormone present, the black rockfish were not as aggressive when put in stress-inducing situations. This is contradictory to the results of our current study. Our results concluded that environmental enrichment did not affect the aggressive behaviors of the Betta fish, and while the black rockfish study looked specifically at cortisol and aggression, it did still find that environmental enrichment decreased aggressive behaviors. There were some differences in methodology between the current experiment and of the study previously mentioned. The first of these major differences was the species used. Different species could be impacted by enrichment differently and thus species-typical differences could have influenced the conflicting conclusions. Another major difference was that the black rockfish were housed in groups. The current study had each fish individually housed in separate tanks prior to, and during, experimentation, unlike the black rockfish experiment that had six tanks containing 40 fish each. The rockfish experiment also consisted of 20 plastic plants in each environmental enrichment tank, in comparison to our two plants; the control tanks were barren in both experiments. Thus, the type of enrichment varied between the current study and the black rockfish study. Finally, in the previous study, the fish were in the environmental enrichment conditions for eight weeks while the fish in the current study were in the environmental enrichment condition for two weeks. The difference in duration of housing in an enriched environment could have impacted the differing outcomes of the studies. It could be that environmental enrichment may not affect aggressive behaviors unless the organism has been exposed to it for a longer period of time, while shorter time frames may not have as great of an impact on behaviors. Therefore, while the results of our study differ from the results of the black rockfish study, this could be because of the different experimental methodology and further research could examine each type of design to analyze the effects of each.

Environmental enrichment and SSRIs have each been shown to have an impact on aggression in organisms when they have been studied separately, yet the research on how the two interact is scarce, and the current study can help fill that gap. Our study looked at the interaction between fluoxetine and environmental enrichment, and if the interaction was significant enough to decrease the levels of aggression in the Betta fish to a greater degree than each treatment separately. While separately the two factors showed an effect on aggression in past studies, when studied together in the present study, environmental enrichment was not shown to have a significant impact on aggressive displays. These findings could be helpful for future studies if they are shown to be repeatable. If further studies show that when together, environmental enrichment and SSRIs are not impacting behavior as originally hypothesized, this could further the research into why this is. Since enrichment has been linked to decreased aggression in past studies when observed by itself, further research could be done to help explain why environmental enrichment may not affect aggression when it is studied with the presence of SSRIs. Varying different parameters in future studies may provoke the thought of how enrichment might lose its influence when SSRIs are added to the environment. Environmental enrichment can be achieved through a variety of manipulations, some of which we did not include in the present study. One type of enrichment manipulation could be the availability of food and levels of predation. With changes in the availability of food and the number of predators in an environment, the aggression levels of the subjects could be impacted as well. This was tested in a previous study on Brachyrhaphis episcopi fish, in populations that are subjected to either high or low predation pressures. In high predation populations, increased aggressive behaviors were observed and the fish were more active compared to low predation populations (Archard and Braithwaite, 2011). In lower predation populations, females had a greater variation in behaviors and spent more time inspecting the mirror. It is thought that females have this variation because of the trade-off between predation and food availability. This shows that each type of enrichment may not always decrease levels of aggression, but rather the type of enrichment will affect aggression. Higher enrichment with increased predation caused higher levels of aggression and lower levels of enrichment caused lower levels of aggression when studied. While it would be expected that increased competition for food would increase levels of aggression, that study shows that how the environment is enriched can change how the aggression levels change. Further research in Betta fish can be done on the relationship between the types of manipulations of the environment and changes in aggression levels. The effect of environmental enrichment is also determined by group size, particularly in fish. Fish that have seen the greatest benefits from environmental enrichment are single housed fish compared to social fish housing (Collymore et al., 2015). In a population of Zebrafish, Danio rerio, which are schooling fish, isolation causes an increase in stress. In comparison to single housed fish, schooling fish did not have a preference between compartments of barren or enriched environments. However, when the schooling fish were held in isolated enclosures, their stress increased indicating that the type of environment the schooling fish are held in is not as impactful as whether the schooling fish are isolated or not. As a result, environmental enrichment can benefit non-schooling fish more than schooling fish. In another study with Zebrafish, environmental enrichment increased aggression (Woodward et al., 2019). Zebrafish are schooling fish where a hierarchy is known among the fish which can lead to territoriality in an enriched environment with more resources that can be defended. In a different study, environmental enrichment had no influence on the schooling fish Serrapinnus notomelas (Alexandre et al., 2020). Increasing the enrichment of tanks caused fish to have increased foraging activities but aggression did not increase. This reveals that enrichment, shoal size, and species differences need to be considered when assessing aggression in captive fish, and this may explain the discrepancies we found between our study and past research.

Future studies could be conducted to examine why enrichment was not a significantfactor in decreasing aggression. Although we had a repeated measures design with thefluoxetine, we did not with the environmental treatment. Also, we had a relatively smallersample size, and our results could have been impacted by that. If our study was repeated with more subjects, aggression levels may have been impacted by the environment the subject was housed in and therefore align with past research. Likewise, if repeated with more subjects, if we extended the time in the environmental treatment, and the conditions repeated more times, this study could further the research on the interaction between environmental enrichment and SSRIs. Little research has been done in the past on how the two interact with each other, so further research on this topic could help explain what causes enrichment to not have a significant effect on aggression like SSRIs do.

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