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Alan Emerson

# Effects of Repeated Systemic Administration of Fluoxetine on Offensive Aggresion in Syrian Hamsters (*Mesocricetus auratus*)

***By Alan Emerson***

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# Effects of Repeated Systemic Administration of Fluoxetine on Offensive Aggresion in Syrian Hamsters (*Mesocricetus auratus*)

By

Alan Emerson, Joseph Terranova, and H. Elliott Albers

| Honors Thesis Director | Honors College Associate Dean |
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| H. Elliot Albers, Ph.D | L. Sarah Cook, Ph.D |
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**Abstract**

Syrian hamsters are a useful model for offensive aggression because males and females spontaneously engage in agonistic bouts. In hamsters, there is a large sex difference on aggression in the serotonin (5-HT) pathways. Male aggression is inhibited and female aggression increases with injections of a 5-HT agonist into the anterior hypothalamus (AH), but little is known if similar effects are seen in adult hamsters with repeated systemic administration of the selective serotonin reuptake inhibitor (SSRI), fluoxetine (FLX), which is one of the few approved pharmacological treatments for mood disorders in children and adolescents. The goal of this study is to determine if repeated intraperitoneal injections of FLX over 30 days in adolescent male and female hamsters has an effect on offensive aggression similar to site specific alterations of the 5-HT system in the AH. Our data suggest that systemic administration of FLX as adolescents over 30 days does not affect offensive aggression in males or females as adults.


| ***Key Words:*** | ***Hamster*** |
|---|---|
| ***Aggression*** | ***Fluoxetine*** |
| ***SSRI*** | ***5-HT (Serotonin)*** |

## Introduction

Selective serotonin reuptake inhibitors (SSRIs) are one of the only FDA approved pharmaceutical treatments for major depressive disorder (MDD) in children and adolescents in the United States (Oberlander and Miller, 2011). Currently, there are six SSRIs approved by the FDA to treat MDD in children: fluoxetine, sertraline, paroxetine, citalopram, escitalopram, and fluvoxamine (NIMH, 2014). Fluoxetine (FLX), however, is currently considered the most effective out of the six (Cheung et at., 2006). Adolescent MDD treatment with FLX needs additional research in part because of the increasing rate of adolescents receiving prescriptions of SSRIs, and the variable effects they have on emotion (Ahn et al., 2011). In the late 90s, the rate of SSRI prescription use between the ages of 7 and 17 increased 10-fold and remains at a relatively consistent level (Zito et al., 2003). There has been shown to be a significant placebo effect in adolescents regarding SSRI treatment (Birmaher et al., 1996), but later reports show that there are cases where adolescents can have higher rates of depressive behaviors, failed suicide attempts, and successful suicide attempts in the first few weeks while taking FLX (Olfson et al., 2006). FLX treatment can also increase hostility in both children and adolescents just weeks after starting a low-dose regiment (Ahn, Yakutis, & Frazier, 2011), but less is known about how FLX taken during adolescents or childhood affects behaviors in adulthood. Much of the FLX literature during human development focuses on the treatment of the depressive effects of MDD and suicide rates, but not the developmental effects related to other areas of sociality, particularly aggression in adulthood. Until recently, there were no data using animal models to show how chronic administration of FLX during periods of development (e.g. adolescence) could influence offensive aggression behaviors in adulthood and the sex differences.

The Syrian hamster (*Mesocricetus auratus*) is a useful animal model for studying offensive aggression because both male and female Syrian hamsters are spontaneously aggressive as opposed to rats or mice that may not aggressively engage with another member of the same sex without experimental manipulations (Terranova et al., 2016). Offensive aggression in Syrian hamsters is well documented and increases significantly with the onset of maturation around adolescents (Schoenfeld & Leonard, 1985). The neural mechanisms underlying Syrian hamster offensive aggression phenotype is, in a large part, mediated by 5-HT and arginine-vasopressin (AVP) systems that converge at the anterior hypothalamus (AH) with reciprocal connectivity from the lateral septum (LS), medial amygdala (MeA), bed nucleus of the stria terminalis (BNST), and ventrolateral hypothalamus (VLH) (Delville, De Vries, & Ferris, 2000). Interestingly, there are significant sex differences in the expression of aggression in hamsters. Microinjection of a 5-HT1a agonist into the AH increases aggression in females and decreases aggression in males, but microinjection of AVP into the same region shows an opposite effect, where female aggression is inhibited and male aggression increases (Terranova et al., 2016). Peripheral administration of a large single FLX dose before an agonistic encounter increases aggression in females but decreases aggression in males (Terranova et al., 2016). However, these data raise more questions when compared to the experiment done by Melloni and Ricci (2013) that show chronic systemic administration of a low-dose of FLX over 30 days in adolescence increases aggression in mature males, which is opposite to the decreases in aggression observed with a single high FLX dose in mature males (Terranova et al., 2016). These data further support that sex differences in the AVP and 5-HT networks contribute to variations in aggression, and the stage of development and dosages of drug treatment are contributing factors.

These findings are not surprising considering that rates of adult human psychiatric disorders have significant sex differences with females having higher occurrences of post-traumatic stress disorder (PTSD), generalized anxiety disorder, MDD, obsessive compulsive disorder, and panic disorders, which increase aggression as a secondary side effect in some individuals (Cover et al., 2014), but there are reports that show higher levels of aggression might act as a protective factor against mood disorders (Cooper at al., 2015). These sex differences in mood disorders have also been observed in adolescents, where females have twice the occurrence of MDD (Hankin et al 1998). Recently, it has been shown that SSRIs have a greater effect on reducing the negative effects of the previously mentioned mood disorders if there are higher levels of estrogens (Gorman, 2006), but the mechanisms for this effect has not been studied. The study by Melloni and Ricci (2013) only used male Syrian Hamsters, so the effects of chronic systemic administration of FLX in adolescent female hamsters on adult aggression is still poorly understood. Answering more questions about the areas of sociality that are affected differentially in males and females could help create specialized clinical treatments between sexes affected by the same disorder, and help understand the long-term side effects of SSRIs. Furthermore, the aim of this study is to investigate the hypothesis that repeated systemic administration of FLX in adolescent female Syrian hamsters will decrease adult aggression.

## Methods

### *Animals and Drug Treatment*

To begin preparations for the experiment, 6 in-house female breeder hamsters were paired up with in-house males. The pups were weaned on postnatal day 25 (P25) into individual poly-carbonated (24 x 43 x 20) cages on a reverse light:dark cycle of 14L: 10D with lights off at 10:00 am. Food and water were given ad libitum. One week before weaning, female and male nonaggressive intruders (NAIs) were ordered from Charles River Laboratories (Wilmington, MA). The NAIs were group housed at 3-5 hamsters per cage in the same housing conditions as the bred experimental hamsters. After birth, 16 male and 32 female pups were randomly selected and assigned into experimental groups. The males were included to be controls to support the data by Malloni and Ricci (2013). The 16 males were assigned to either vehicle injections of saline (n=8) or low-dose FLX (0.3 mg/kg, n=8). The 32 females were assigned to one of four groups (n = 8 per group). The conditions were vehicle, low-dose (0.3 mg/kg), medium dose (0.7 mg/kg), or high-dose (1.0 mg/kg) of FLX. The FLX solutions were prepared using sterile saline and fluoxetine hydrochloride. The mass of each experimental hamster was taken at 3-day intervals starting on the first day of injections (P27) to test the effects of the different dosages on weight gain. The i.p. injections were administered each day in a circular pattern around the abdomen after washing the area with a sterile alcohol swab to reduce the change of peritonitis. All injections were administered with 0.5-gauge insulin needles. The last injection was done on P57 for all groups. All experiments were conducted in accordance with the *National Institutes of Health Guidelines for the Use of Animals* (2011) and were approved by the Georgia State University Institutional Animal Care and Use Committee.

*Behavioral Testing*

One week before behavioral testing began, the experimental and NAI females were monitored for their estrous stage to ensure the treatment females selected for behavioral testing were in diestrus, when they show the most offensive aggression, and that the NAIs were in proestrus, when they show the lowest aggression without lordosing (Floody and Pfaff, 1977). Behavioral testing began following the last day of injections (P58) at 10:00 when the dark cycle began. A variation in the resident/intruder model described by Terranova and associates (2016) was used to test offensive aggression. Briefly, the experimental and NAI hamster of the same sex and similar weight were placed simultaneously into a neutral cage. The agonistic bouts were recorded for 10 minutes, then the videos were scored for duration of total time (s) exhibiting offensive behaviors that included: upright aggressive posture, aggressive pursuits, attacks, bites, and pins. The latency to attack was also scored, and is quantified as the time the test began to when the treatment hamster initiated the first attack and attempted to bite. A score of 600 seconds was given if the treatment hamster did not initiate an attack. Statistical analysis was performed using SPSS comparing the mean attack latency and aggression of the males and a separate analysis of the females.

**Results**

A two-tailed t-test was done to compare the saline and low-dose treated males, and the saline, low, medium, and high dose females. There was no effect in aggression between males treated with low-dose FLX or saline ($t(6) = 0.400$; $p = .700$; Fig 1a), and there was a trend of low-dose treatment towards a significant decrease in latency to attack ($t(6) = 1.49$; $p = .17$; Fig 1b). A one-way ANOVA analysis for the females showed no significant effect of any treatment

on aggression ($p = .38$; Fig 2a.) or latency to attack ($p = .90$; Fig 2b.). Fisher's Least Significant Difference post-hoc analysis was run to compare all treatment groups for females, and found no significant differences.

**Discussion**

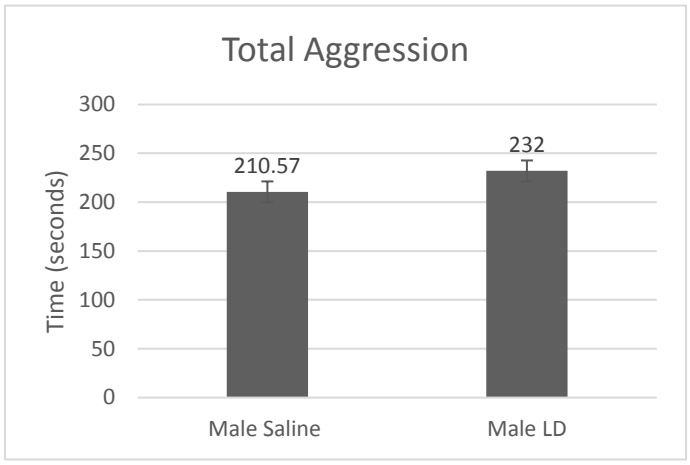
There are conflicting reports that show SSRIs, primarily fluoxetine, administered to youth can have detrimental or no effect. Some report increased aggression, suicide attempts, violence, and worse depression symptoms (Ahn et al., 2011), but others show no changes in social behaviors in youth (Burghardt et al., 2004). These data show that repeated systemic injection of varying dosages of fluoxetine as adolescents do not have an effect on total aggression time in males or females when they are adults, which does not support the previous work by Melloni and Ricci (2013). However, Melloni and Ricci (2013) evaluated individual aspects of aggression instead of grouped into one category. Future experiments would separate the aggressive behaviors into distinct categories. Flank marking may also be included, as its role in expression of aggression and dominance is mediated by the AH, which contains 5-HT inputs in males and females (Ferris et al., 1996).

A possible explanation for the results could be the systemic administration as opposed to direct microinjection into the AH or innervating nuclei. Because the FLX treatment was capable of traveling to all areas of the brain, it is possible that regions in the brainstem, limbic, and cortical areas that suppress aggression could have been altered in such a way to reduce any effects that would have increased aggression, which could be explored because previous reports have shown that FLX treatment increases afferent connections in those regions (Zhou et al., 2006). Exploring the changes in 5-HT afferent and efferent connections, spine density, and the various 5-HT receptors in the AH, LS, BSNT, MeA, and VLH would provide further

mechanisms of FLX changes in the aggression pathways when FLX is administered systemically.

Another confounding variable is the mode of administration. Chronic physical abuse or uncontrollable stressors in early life have been shown to alter expression and susceptibility to aggression in several rodent models and humans (Chistiakov and Chekhonin, 2017). Although many efforts were done to reduce infection and damage to visceral organs, it could be that some hamsters had developed peritonitis after the repeated injections, which reduces locomotion and could influence aggression.

In summary, the results on male aggression did not match the previously done experiment by Melloni and Ricci (2013), but further examination of the results is needed. Various factors such as the systemic application and mode of injection could impact the observed behaviors. With the conflicting data on how SSRIs differentially affect children and adolescents with MDD, it is important to evaluate various animal models, SSRIs, and dosages to reflect homologous treatments in humans to enhance our understanding of pharmacological treatments and how they could affect long-term development.



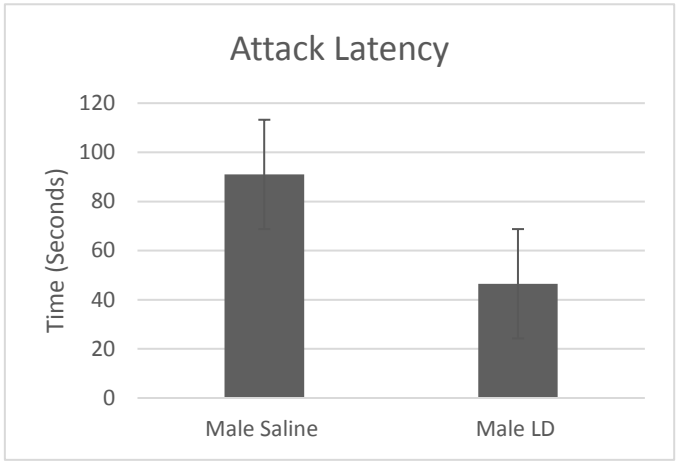
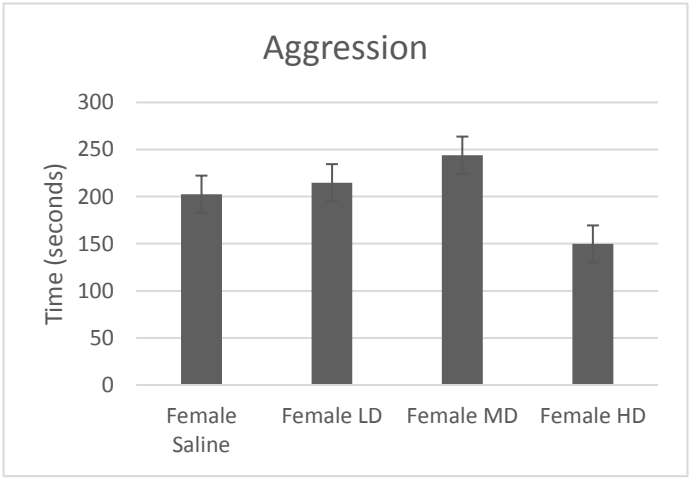




*Figure 1.* Effects in males of fluoxetine administration during adolescents on (a) total aggression and (b) attack latency in adulthood. Low-dose (0.3 mg/kg) exposure had no effect on total aggression or attack latency ($p>0.05$)



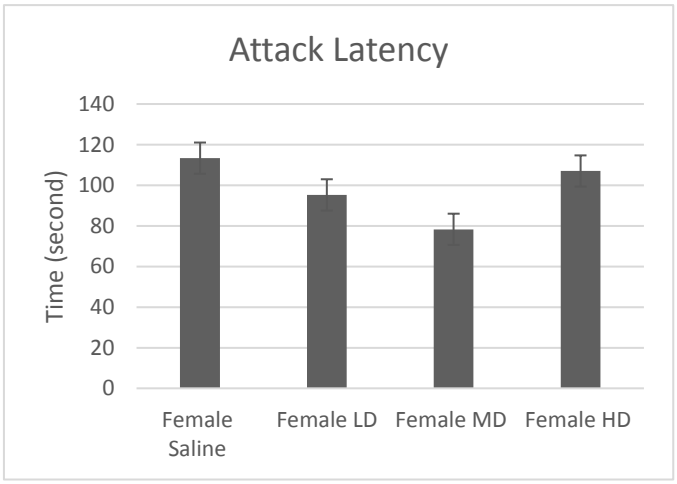




*Figure 2.* Effects in females of fluoxetine administration during adolescents on (a) total aggression and (b) attack latency in adulthood. Low-dose (0.3 mg/kg), medium-dose (0.7 mg/kg), and high-dose (1.0 mg/kg) exposure had no effect on total aggression or attack latency ($p>0.05$)

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