5-7-2016

# Sex Differences in the Effect of Prenatal and Perinatal Fluoxetine Exposure on Adult Aggression and Avoidance in the Syrian Hamster

Ryan J. Slaby

# SEX DIFFERENCES IN THE EFFECT OF PRENATAL AND PERINATAL FLUOXETINE EXPOSURE ON ADULT AGGRESSION AND AVOIDANCE IN THE SYRIAN HAMSTER

An Honors Thesis

Submitted in Partial Fulfillment of the

Requirements for Graduation with

Undergraduate Research Honors

Georgia State University

2016

by

Ryan Joseph Slaby

Committee:

_____

Dr. H. Elliott Albers , Honors Thesis Director

_____

Dr. Sarah Cook, Honors College Associate Dean

_____April 25, 2016_____

Date

SEX DIFFERENCES IN THE EFFECT OF PRENATAL AND PERINATAL FLUOXETINE EXPOSURE ON ADULT AGGRESSION AND AVOIDANCE IN THE SYRIAN HAMSTER

by

RYAN JOSEPH SLABY

Under the Direction of Elliott H. Albers, Ph.D

## ABSTRACT

Anti-depressants are commonly used to treat major depression and post-traumatic stress disorder. 17% of women experience major depression during pregnancy, where up to 10% of pregnant women use antidepressants. 20% these women use Prozac (fluoxetine) to treat major depressive symptoms, which crosses the placental barrier and is present in breast milk. Little is known about how exposure to developmental fluoxetine affects adulthood behaviors such as agonistic and submissive behaviors, especially in females. Furthermore, the effects of developmental fluoxetine exposure on aggression and avoidance in Syrian hamsters have not been studied. Therefore, we explored how prenatal and perinatal exposure to fluoxetine affects adulthood aggression and avoidance in male and female Syrian hamsters. Dams were given fluoxetine via drinking water 7 days prior impregnation. Fluoxetine administration continue until offspring reached postnatal day (PD) 12. The offspring were weaned and group-housed at PD 25 and single-housed at PD 60. Animals were handled one week prior to behavioral testing. The following week, animals were tested for aggression in a neutral arena with a non-aggressive stimulus hamster of the same sex. Another group of hamsters were tested for avoidance behavior in a neutral arena 24 hours after social defeat. Duration of aggression and avoidance were

quantified. There was no main effect of sex or drug nor was there an interaction. Therefore, we reject our hypothesis of prenatal and perinatal exposure to fluoxetine will affect aggression and avoidance in male and female Syrian hamsters. These findings may be due to a ceiling effect in Syrian hamsters, where the subtle effects of developmental fluoxetine exposure were not observable.

INDEX WORDS: Syrian Hamster, Aggression, Avoidance, Fluoxetine, SSRI, Prenatal, Perinatal, Serotonin, Pregnancy, Social Behavior, Mother

# SEX DIFFERENCES IN THE EFFECT OF PRENATAL AND PERINATAL FLUOXETINE EXPOSURE ON ADULT AGGRESSION AND AVOIDANCE IN THE SYRIAN HAMSTER

by

RYAN JOSEPH SLABY

An Honors Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Bachelors of Neuroscience

in the College of Arts and Sciences

Georgia State University

2016

# SEX DIFFERENCES IN THE EFFECT OF PRENATAL AND PERINATAL FLUOXETINE EXPOSURE ON ADULT AGGRESSION AND AVOIDANCE IN THE SYRIAN HAMSTER

by

RYAN JOSEPH SLABY

Honors Thesis Director: Elliott H. Albers, Ph.D
Honors College Associate Dean: Dr. Sarah Cook

Electronic Version Approved:

GSU Honors College
Georgia State University
Date:

TABLE OF CONTENTS

# ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Elliott Albers for support and funding this project. I would also like to acknowledge Joseph Terranova and Alisa Norvelle for technical assistance and guidance throughout this research project. Thank you for all that you do and have done. This work is supported by NSF Grant IOS-0923301.

# LIST OF FIGURES

# INTRODUCTION

Anti-depressants are commonly used in the pharmacological treatment for affective disorders such as major depressive order and post-traumatic stress disorder (Melartin et al., 2005 & Bisson, 2010), where 11% of Americans over the age of 12 use anti-depressants (Pratt et al., 2011). One of the most common types of anti-depressants prescribed are selective serotonin reuptake inhibitors (SSRIs), where fluoxetine (Prozac) is widely used amongst depressed individuals (Ferguson, 2001). Maternal depression affects 17% of women (Alwan et al., 2011), where 10% of pregnant women use antidepressants to treat depressive disorders (Cooper et al., 2007). However, approximately 20% of pregnant women currently taking antidepressants specifically take fluoxetine (Cooper et al., 2007). Because fluoxetine crosses the placental barrier and is present in mother's breast milk (Rampono et al., 2009 & Kim et al., 2006), it may affect the development of a mother's offspring. Neuronal growth employs serotonin for a number of neurodevelopmental processes, such as cone motility (Vitalis et al., 2003 & Gaspar et al., 2003). If serotonin levels are increased synaptically, then neurodevelopment may alter and affect offspring behavior. Give the number of women using SSRI's, particularly fluoxetine during and throughout pregnancy, the prenatal and perinatal effects on the development of the mother's offspring is an important area for investigation.

There have been a number of human and non-human studies regarding fluoxetine's effects on development. Mother's taking fluoxetine throughout pregnancy have children who experience an increased risk for low weight and premature birth (Chambers et al., 1996). Rodent studies also demonstrate a lower birth rate with prenatal fluoxetine exposure (Oliver et al., 2011). Furthermore, children exposed prenatally to fluoxetine experience social withdrawal, anxiety and

depression (Oberlander et al., 2010). In both human (Oberlander et al., 2005) and rat studies (Hansen et al. 1997), offspring prenatally exposed to fluoxetine experience shorter recovery time to pain. In addition, rats demonstrate reduced play behavior and conspecific interactions with prenatal fluoxetine exposure (Hansen et al., 1997).

Fluoxetine exposure affects aggression in rodents throughout various developmental stages. Adolescent fluoxetine treatment increases aggression and the number of serotonergic afferent projections responsible for aggressive behavior in Syrian hamsters (Ricci et al., 2012). Adult male rats show increased foot-shock induced aggression when exposed prenatally to fluoxetine and in comparison to other drugs (Singh et al., 1998). Prenatal exposure to fluoxetine increases adulthood aggression, improves spatial memory, and reduces anxiety-like behavior in male mice (Svirsky et al. 2016 & Kiryanova et al., 2014). To the effects of fluoxetine on aggression, we explored the effects of prenatal and perinatal fluoxetine exposure on aggression in Syrian hamsters.

In addition to aggression, social defeat results in increased avoidance and anxiety behaviors in rodents. Serotonin signaling pathways play a major role in the effect of social defeat on behavior, where avoidance and anxiety behaviors result from social defeat in Syrian hamsters (Clinard et al. 2015 & Badder et al. 2014). The expression of condition defeat after social defeat is modulated and mediated by serotonergic pathways in Syrian hamsters (Badder et al., 2014 Harvey et al., 2012). Fluoxetine treatment affects the anxiety behaviors induced by social defeat. Fluoxetine pre-treatment before social defeat prevents reduced food intake and body weight in male rats (Berton et al., 1999). Continuous social defeat reduces locomotor activity and exploration in rats, where social defeat paired with fluoxetine treatment reduces these effects

(Rygula et al., 2006). Adolescent fluoxetine treatment in mice increases adulthood social interaction after social defeat and increases adulthood anxiety as measured by the elevated-plus maze (Iñiguez et al., 2014). Because social defeat is regulated by serotonergic pathways and its negative effects on behavior were reduced by fluoxetine treatment, we explored the effects of prenatal and perinatal fluoxetine exposure in regards to social defeat in Syrian hamsters.

Most rodent research regarding the effects of fluoxetine on behavior focuses on male mice and rats. However, Syrian hamsters are a robust model for studying serotoninergic drugs on social behavior, such as aggression, anxiety, avoidance, and communicative behavior (Morrison et al., 2015; Albers et al., 2002; Cooper et al., 2012). Therefore, we asked how prenatal exposure to fluoxetine modulates adulthood aggression and avoidance in male and female Syrian hamsters. In order to address the issue of women regularly consuming fluoxetine before, during, and after pregnancy, dams were given fluoxetine 1 week before impregnation, up until their offspring were weaned postnatal day (PD) 12. Fluoxetine is administered orally in humans; therefore, administering fluoxetine orally in the dams is more translatable and relevant to human consumption. We chose to administer fluoxetine orally through drinking water, instead of I.P injections, because stressing the dams may lead to a possible confounds in our experiment (Ryabinin et al., 1999).

# MATERIALS and METHODS

12 adult female Syrian hamsters were designated to be the dams, and were single-housed throughout this study. 6 dams were given fluoxetine (20 mg/kg) via drinking water 7 days before pregnancy, and 6 dams were given regular tap water (vehicle control). After monitoring the dam's reproductive cycle, they were impregnated during the estrus. Approximately 36 pups (male = 18; female = 18) were from fluoxetine treated dams, and 38 pups (male = 19; female = 19) were from the vehicle control dams. The dams continued fluoxetine treatment until postnatal day (PD) 12, because pups began to drink and eat outside the mother's breastmilk. We did not want the pups to directly consume fluoxetine from the drinking water, because this would have been a serious confound.

Experimental animals were weaned at PD 25 and housed in same sex groups of 4 to 6 with others from the same treatment group (fluoxetine exposure or tap water vehicle). At PD 60, experimental animals were single-housed. Two weeks after handling, the offspring were handled from PD 74- 81. Handling included monitoring the reproductive cycle of females, and allowing both males and females to become accustomed to human interaction. All female experimental animals were tested during the Diestrus phase of the estrus cycle.

**Testing:**

Aggression and avoidance testing occurred from PD 81-85, where 4 groups for each test (vehicle male, vehicle female, prenatal fluoxetine male, prenatal fluoxetine female) contained 5 -7 hamsters. Aggression testing consisted of a neutral arena, where the experimental animal was paired with a non-aggressive stimulus hamster of the same sex. Both animals were placed in the

neutral arena and the duration of aggression for the experimental animal was quantified across the 5 minute test.

Avoidance testing consisted of a 15 minute social defeat, where the experimental animal was paired with a resident aggressor (RA) in the RA's cage. 24 hours after social defeat, the experimental animal was placed in a neutral arena with an unfamiliar RA for 5 minutes. The RA was placed inside a caged mesh box, which laid in the bottom right corner of the arena. The experimental animal was placed in the opposite side of the neutral arena. Avoidance was measured by the duration of time the experimental animal spent on the opposite side of the neutral arena from the RA.

**Statistical Analysis:**

Data was analyzed using IBM SPSS Statistics for Windows. A 2x2 (Sex x Drug Treatment) analysis of variance (ANOVA) was used to ascertain differences between the groups for aggression and avoidance.

# RESULTS

**Aggression:**

We hypothesized that aggression will increase for male and female hamsters prenatally exposed to fluoxetine. Duration of aggression from a one-time 5 minute encounter in adults prenatally exposed to fluoxetine or vehicle control were quantified. No significant main effects of sex ($F(1,21) = 0.462$; $p > 0.05$) or drug ($F(1,21) = 0.023$; $p > 0.05$) were observed. No interaction between sex and drug was observed ($F(1, 21) = 0.868$; $p > 0.05$).

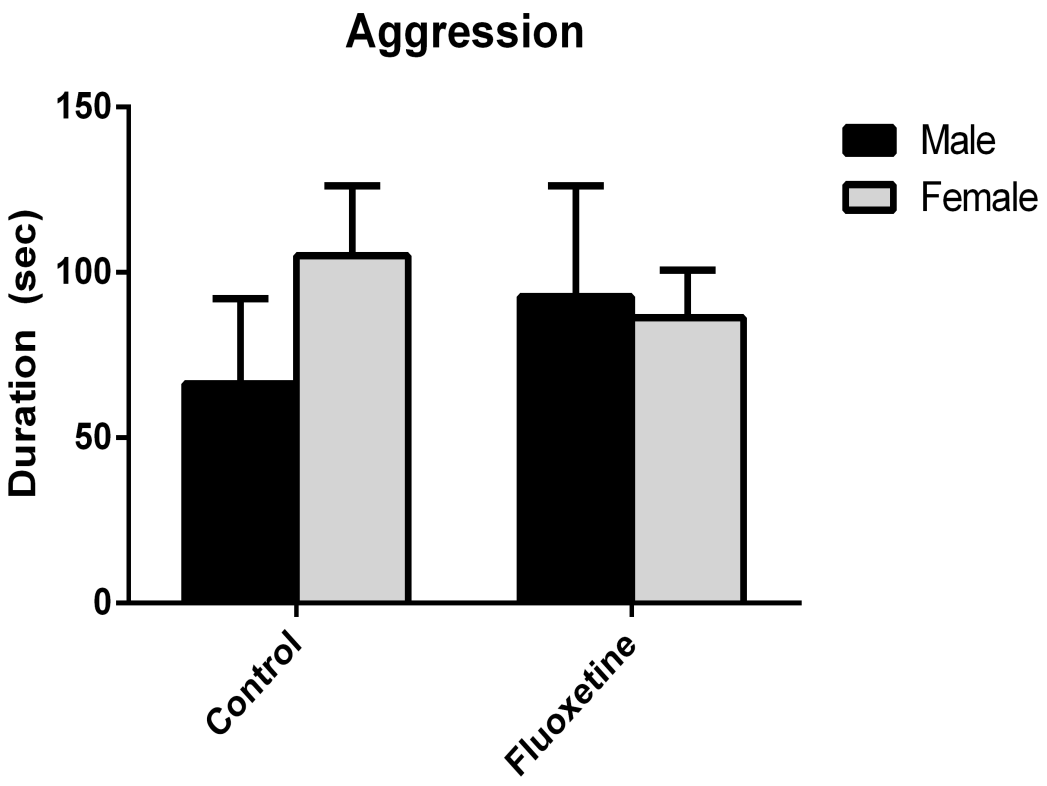


FIGURE 1. Duration of aggression for vehicle control and fluoxetine treated animals.

**Avoidance:**

We hypothesized that avoidance will increase for male and female Syrian hamsters prenatally exposed to fluoxetine. Hamsters were subjected to a 15 minute social defeat experience and were tested for avoidance 24 hours later in a neutral arena with an unfamiliar stimulus animal. Time spent avoiding the stimulus animal was quantified. No significant main effects of sex ($F(1,15) = 0.384$; $p > 0.05$) or drug were observed ($F(1,15) = 0.023$; $p > 0.05$). No interaction between sex and drug was observed ($F(1,15) = 0.375$; $p > 0.05$).

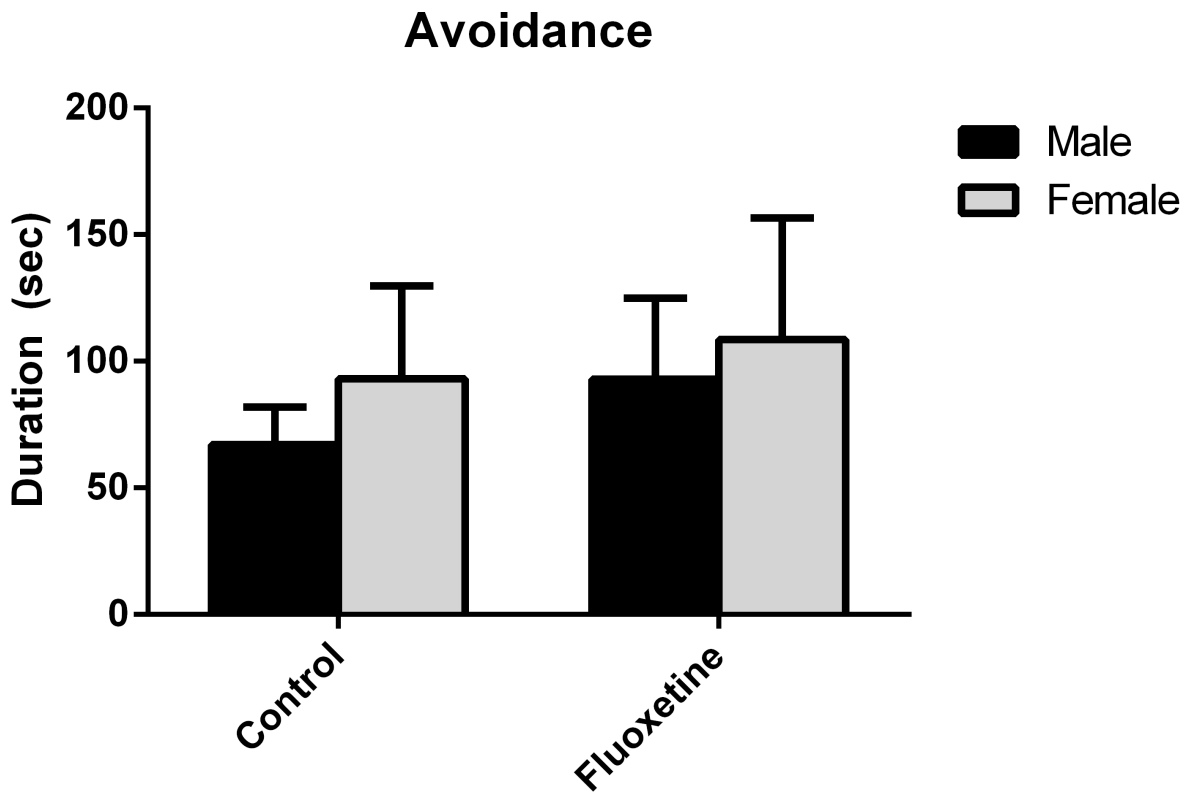


FIGURE 2. Duration of avoidance for vehicle control and fluoxetine treated animals.

# DISCUSSION

There was no main effects of sex and drug, nor was there an interaction of sex and drug for both aggression and avoidance. Thus, our hypothesis that prenatal and perinatal exposure to fluoxetine in male and female Syrian hamsters affects avoidance and aggression has been refuted. We were surprised that developmental exposure to fluoxetine had no effect on adulthood agonistic behaviors, because adolescent fluoxetine exposure increases aggression in adult male Syrian hamsters through modulation of serotonergic and vasopressinergic systems (Ricci et al., 2012). However, the effects of prenatal and perinatal exposure to fluoxetine may be too subtle to modulate adulthood agonistic behaviors. Syrian hamsters are a robust model for agonistic behavior, because they spontaneously display territorial and aggressive behaviors towards other conspecifics (Morrison et al., 2015; Albers et al., 2002; Cooper et al., 2012). In comparison to other rodent species, Syrian hamsters are more territorial and aggressive (Payne & Swanson, 1970; Lerwill & Makings, 1971). Thus, we may have encountered a ceiling effect where the effects of prenatal and perinatal exposure to fluoxetine were too subtle to alter adulthood aggression. The subtle developmental effects of fluoxetine may be more apparent in mice and rats than in Syrian hamsters.

In addition to high levels of agonistic behaviors, Syrian hamsters have a higher metabolism than rats and mice (Schulze et al. 1996). The Syrian hamster dams may have metabolized fluoxetine at faster rates than rat and mouse dams, thus diminishing the developmental effects of prenatal and perinatal fluoxetine on offspring in hamsters. This variance across species calls into question the translation of rodent research for humans, where how rodents experience a drug may differ from how humans experience it.

With regards to avoidance behavior, adolescent and adulthood fluoxetine treatment in rats and mice is associated with decreased avoidance and anxiety behaviors after social defeat (Berton et al., 1999; Rygula et al., 2006). However, no differences regarding avoidance behavior were observed in Syrian hamsters prenatally and perinatally exposed to fluoxetine. Because Syrian hamsters are affected by adolescent fluoxetine exposure (Ricci et al., 2012), and adolescent and adulthood fluoxetine exposure affects avoidance and anxiety behavior in rats and mice (Berton et al., 1999; Rygula et al., 2006), there may be effects of adolescent and adulthood exposure to fluoxetine on avoidance behavior for the Syrian hamster. Further research regarding the effects of adolescent and adulthood fluoxetine exposure on the Syrian hamster's avoidance behavior should be pursued.

Prenatal and perinatal exposure to fluoxetine in humans show increased anxiety, depression, premature birth, and other negative effects (Chambers et al., 1996; Oberlander et al., 2010 & Oberlander et al., 2005). In addition, mouse and rat studies show negative impacts in regards to prenatal and perinatal fluoxetine exposure (Oliver et al., 2011; Hansen et al., 1997; Svirsky et al., 2016; Kiryanova et al., 2014; Singh et al., 1998). The investigation of fluoxetine's developmental impacts on behavior and cognition should continue in rats and mice; however, inconclusive results may reoccur in research with the Syrian hamster model. Although the Syrian hamster is a robust model in social behavior research (Morrison et al., 2015; Albers et al., 2002; Cooper et al., 2012), the effects of developmental fluoxetine exposure should be explored in other rodent species.

# REFERENCES

Albers HE, Karom M, Smith D. (2002). Serotonin and vasopressin interact in the hypothalamus to control communicative behavior. Neuroreport, 13: 931–933.

Alwan S, Reefhuis J, Rasmussen SA, & Friedman JM. (2011). "Patterns of antidepressant medication use among pregnant women in a United States population." Journal of Clinical Pharmacology, 51(2): 264–270.

Badder LR, Carboni JD, Burleson CA, Cooper MA. (2014). "5-HT1A receptor activation reduces fear-related behavior following social defeat in Syrian hamsters." Pharmacol Biochem Behav, 122: 182–190.

Berton O, Durand M, Aguerre S, Mormède P, Chaouloff F. (1999). "Behavioral, neuroendocrine and serotonergic consequences of single social defeat and repeated fluoxetine pretreatment in the Lewis rat strain." Neuroscience, 92: 327–341.

Bisson JI. (2010). "Post-traumatic stress disorder." BMJ Clin Evid, 1005.

Clinard CT, Bader LR, Sullivan MA, Cooper MA. (2015). "Activation of 5-HT2a receptors in the basolateral amygdala promotes defeat-induced anxiety and the acquisition of conditioned defeat in Syrian hamsters." Neuropharmacology, 90:102–112.

Cooper WO, Willy ME, Pont SJ, & Ray WA. (2007). “Increasing use of antidepressants in pregnancy.” American Journal of Obstetrics & Gynecology, 196(6): 544, e541–e545.

Ferguson JM. (2001). “SSRI Antidepressant Medications: Adverse Effects and Tolerability.” Prim Care Companion J Clin Psychiatry, 3: 22–27.

Gaspar P, Cases O, Maroteaux L. (2003). “The developmental role of serotonin: news from mouse molecular genetics.” Nat Rev Neurosci, 4: 1002–1012.

Hansen HH, Sanchez C, & Meier E. (1997). “Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immo- bility in adult rats: A putative animal model of depression.” The Journal of Pharmacology and Experimental Therapeutics, 283: 1333–1341.

Harvey ML, Swallows CL, Cooper MA. (2012). “A double dissociation in the effects of 5-HT2A and 5-HT2C receptors on the acquisition and expression of conditioned defeat in Syrian hamsters.” Behavioral Neuroscience, 126(4): 530–7.

Iñiguez S. D. *et al* (2014). “Fluoxetine exposure during adolescence alters response to aversive stimuli in adulthood.” J. Neurosci, 34:1007–1021.

Kim J, Riggs KW, Misri S, Kent N, Oberlander TF, Grunau RE, Fitzgerald C, Rurak DW. (2006). "Stereoselective disposition of fluoxetine and norfluoxetine during pregnancy and breast-feeding." Br J Clin Pharmacol, 61: 155-163.

Lerwill CJ, Makings P. (1971). “The agonistic behavior of the golden hamster Mesocricetus auratus (Waterhouse)”. Anim. Behav, 19: 714– 721.

Melartin TK, Rytsälä HJ, Leskelä US, Lestelä-Mielonen PS, Sokero TP, Isometsä ET. (2005). "Continuity is the main challenge in treating major depressive disorder in psychiatric care.” J Clin Psychiatry, 66: 200-207.

Morrison T, Ricci L, Melloni R. (2015). “Aggression and anxiety in adolescent AAS-treated hamsters: A role for 5HT3 receptors”. Pharmacol Biochem Behav, 134: 85–91.

Oberlander TF, Grunau RE, Fitzgerald C, Papsdorf M, Rurak D, & Riggs W. (2005). “Pain reactivity in 2-month- old infants after prenatal and postnatal serotonin reuptake inhibitor medication exposure.” Pediatrics, 115(2): 411–425.

Oberlander TF, Papsdorf M, Brain UM, Misri S, Ross C, & Grunau RE. (2010). “Prenatal effects of selective serotonin reuptake inhibitor antidepressants, serotonin transporter promoter genotype (SLC6A4), and maternal mood on child behavior at 3 years of age.” Arch Pediatr Adolesc Med, 164(5): 444–451.

Payne AP, Swanson HH. (1970). “Agonistic behaviour between pairs of hamsters of the same and opposite sex in a neutral observation area.” Behaviour, 36, 260–269.

Pratt LA, Brody DJ, Gu Q. (2011). "Antidepressant Use in Persons Aged 12 and Over: United States, 2005-2008." NCHS Data Brief No. 76. Hyattsville, MD: National Center for Health Statistics.

Rampono J, Simmer K, Ilett KF, Hackett LP, Doherty DA, Elliot R, Kok CH, Coenen A, Forman T. (2009). “Placental transfer of SSRI and SNRI antidepressants and effects on the neonate.” Pharmacopsychiatry, 42: 95-100.

Ricci LA, Melloni RH. (2012). "Repeated Fluoxetine Administration During Adolescence Stimulates Aggressive Behavior and Alters Serotonin and Vasopressin Neural Development in Hamsters." Behavioral Neuroscience, 126: 640-653.

Ryabinin AE, Wang YM, Finn DA. (1999). “Different levels of Fos immunoreactivity after repeated handling and injection stress in two inbred strains of mice.” Pharmacol Biochem Behav, 63: 143–151.

Rygula R, Abumaria N, Domenici E, Hiemke C, Fuchs E. (2006). “Effects of fluoxetine on behavioral deficits evoked by chronic social stress in rats.” Behav. Brain Res, 174: 188–192.

Schulze J, Schläger W, Wilnsch R, Richter E. (1996). “Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by hamster, mouse and rat intestine: relevance of species differences.” Carcinogenesis, 17 (5): 1093-1099.

Singh Y, Jaiswal AK, Singh M, Bhattacharya SK. (1998). "Effect of prenatal diazepam, phenobarbital, haloperidol and fluoxetine exposure on foot shock induced aggression in rats." Indian Journal of Experimental Biology, 36: 1023-1024.

Svirsky N, Levy S, Avitsur R (2016). “Prenatal exposure to selective serotonin reuptake inhibitors (SSRI) increases aggression and modulates maternal behavior in offspring mice”. Dev Psychobiol, 58(1): 71-82.

Vitalis T, Parnavelas JG. (2003).“The role of serotonin in early cortical development.” Dev Neurosci, 25: 245–256.