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Fluoxetine Exposure Results in Decreased Sensitivity to Cocaine and Sucrose Later in Life: A Study in Female C57BL/6 Mice

Francisco Javier Flores Ramirez
University of Texas at El Paso, fjfloresram@miners.utep.edu

FLUOXETINE EXPOSURE RESULTS IN DECREASED SENSITIVITY TO COCAINE AND SUCROSE LATER IN LIFE: A STUDY IN FEMALE C57BL/6 MICE

FRANCISCO JAVIER FLORES RAMIREZ

Master's Program in Experimental Psychology

APPROVED:

Sergio D. Iñiguez, Ph.D., Chair

Edward Castañeda, Ph.D.

Kristin L. Gosselink, Ph.D.

Laura E. O'Dell, Ph.D.

Charles Ambler, Ph.D.
Dean of the Graduate School

Dedication

To my Buttercup.

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by

FRANCISCO JAVIER FLORES RAMIREZ, B.A.

THESIS

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Abstract

Preclinical evidence indicates that exposure to psychotropic medications, during early development, results in long-lasting altered responses to stress- and reward-related stimuli. However, these animal studies have been conducted, primarily, using male subjects. This is surprising, given that clinical data suggests that females have a higher likelihood, than their male counterparts, to be diagnosed with mood-related illnesses, and thus, be prescribed with psychotropic medications, mostly antidepressants. Therefore, to examine whether enduring reward-related alterations are exhibited as a result of antidepressant exposure, in female subjects specifically, we exposed C57BL/6 female mice to fluoxetine (FLX; 250 mg/l in their drinking water). Specifically, separate groups of mice were exposed to FLX for 15 consecutive days, either during adolescence (postnatal day [PD] 35-49) or adulthood (PD70-84). Twenty-one days later, the mice were examined on their behavioral reactivity to cocaine (0, 2.5, 5, 7.5 mg/kg) using the condition place preference paradigm, or assessed on the 2-bottle choice sucrose (1%) test. Our results indicate that, regardless of age of antidepressant exposure, female mice pre-exposed to FLX displayed reliable conditioning to the cocaine-paired compartment in a dose-dependent manner. However, when compared to respective age-matched controls, antidepressant pre-exposure decreased the magnitude of conditioning at the 5 and 7.5 mg/kg cocaine doses. Furthermore, FLX pre-exposure reduced sucrose preference, without altering total liquid intake. Collectively, the data suggest that pre-exposure to FLX, during adolescence or adulthood, results in a prolonged decrease in sensitivity to the rewarding properties of both natural and drug rewards, in female C57BL/6 mice.

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Chapter 1: Introduction

Major depressive disorder (MDD) is a severe and debilitating illness that affects millions of people across the globe (Ferrari et al., 2013). The prevalence of this disorder is particularly high within the juvenile population, given that up to 11% of adolescents are diagnosed with MDD (Costello et al., 2002). This is problematic because, if untreated, depressed adolescents display higher suicide attempts (Miranda & Shaffer, 2013), engage in illicit drug use (Copeland et al., 2009), and become involved in other risky behaviors (Duell et al., 2017). Currently, the most prescribed pharmacotherapeutic agent for the management of juvenile MDD is the selective serotonin reuptake inhibitor (SSRI), fluoxetine (FLX) – as other antidepressants do not consistently ameliorate depressive symptomology within this population (Emslie & Judge, 2000). As a result, there has been a significant increase in the prescription rates of FLX in individuals within their teenage years. This is surprising, given the pharmacodynamic differences, across numerous psychotropic agents, are commonly reported between developing and adult organisms (Iñiguez, Cortez, Crawford, & McDougall, 2008; Scalzo & Spear, 1985), as well as a dearth of preclinical studies that have assessed for potential long-lasting side effects (Izquierdo et al., 2016; Olivier, Blom, Arentsen, & Homberg, 2011).

Exposure to antidepressants during adolescence, specifically, is concerning because preclinical studies suggest that there are indeed long-lasting neurobiological changes that may alter normal functioning. For example, adolescent FLX exposure has been shown to mediate a prolonged increase in sensitivity to anxiogenic stimuli (Homberg et al., 2011; Karpova, Lindholm, Pruunsild, Timmusk, & Castren, 2009), induce impairments in spatial memory performance (Sass & Wortwein, 2012), and increase the incentive valence of both natural (Iñiguez et al., 2010a) and drug rewards (Iñiguez et al., 2015). Collectively, this complex

behavioral profile may suggest that juvenile FLX exposure mediates a phenotype indicative of altered drug-seeking behavior in adulthood. Nevertheless, it should be noted that the majority of these preclinical studies have been conducted using male subjects as a model system. This is an unexpected experimental approach, given that clinical data suggests that females are more likely than males to be diagnosed with a mood disorder across their lifetime (Kessler, 2003), and thus, are more likely to be prescribed with FLX (Hoffmann et al., 2014). To address this gap in the literature, the purpose of this investigation is to assess the prolonged effects of adolescent FLX exposure on the incentive motivation for cocaine and sucrose, in female C57BL/6 mice.

Specific Aims of Master's Thesis:

Aim 1: Examine the prolonged effects of adolescent fluoxetine exposure on the sensitivity to the rewarding properties of cocaine and sucrose, later in life in female C57BL/6 mice.

Aim 2: Examine whether any changes observed are dependent on the age of exposure to fluoxetine.

Hypotheses: Previous work in male C57BL/6 mice has shown that exposure to FLX during adolescence leads to enduring *increases* in sensitivity to the rewarding properties of cocaine, as well as sucrose, later in life (Iñiguez et al., 2015). Thus, for this project, our central hypothesis is that juvenile FLX exposure will also change responses to cocaine and sucrose, when tested in adulthood, in female C57BL/6 mice.

Chapter 2: Methods

Animals

Adult and adolescent Female C57BL/6 mice were utilized in the current investigation (Charles River, Hollister, CA). Mice were housed in polypropylene cages (3-4 per cage), which were bedded with wood shavings, and had access to food and water *ad libitum*. The colony room was maintained at a temperature between 21-23 C°, under a 12-h light/dark cycle (lights on at 700 h). In rodents, adolescence is a period of development that ranges from PD21 to PD60 that is defined by the surge of adrenal androgens/neuroactive steroids, as well as increases in social and playful interactions (Laviola, Macri, Morley-Fletcher, & Adriani, 2003; Spear, 2003) – characteristics that are similar in humans during adolescence. Furthermore, adolescence is a period that is also characterized by changes in brain plasticity (Spear, 2003), such as increases in pruning, as well as changes in the serotonergic system (Airan et al., 2013). Serotonin plays a fundamental role in the regulation of mood and anxiety, as well as impulse control and arousal, which are behaviors/syndromes that underlie affect-related illnesses. Interestingly, when compared to adults, decreases of serotonin take place during early development, which are correlated with decreases in impulse control as the brain matures during the juvenile stage of development (Arain et al., 2013). As such, any pharmacological insult to the serotonergic system, during adolescence specifically, may result in altered behavioral changes in adulthood. All studies were approved by the institutional animal care and use committee at The University of Texas at El Paso, and conformed to National Institutes of Health guidelines.

Drugs

Fluoxetine hydrochloride (FLX) was purchased from Spectrum Chemicals (Gardena, CA) and was dissolved (250 mg/l) in sterile double distilled water (vehicle; VEH). FLX was delivered

ad libitum in the drinking water (changed weekly) in light protected bottles (Ancare, Bellmore, NY; Model PC9RH8.5RD). The dose of FLX was selected because it yields a dosage close to 25 mg/kg (Dulawa, Holick, Gundersen, & Hen, 2004) – taking into account that females and adolescents metabolize FLX faster than males and/or adults (Anderson, 2005; Hodes, Hill-Smith, Suckow, Cooper, & Lucki, 2010; Wegerer *et al*, 1999). Cocaine hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO) and was diluted with sterile 9% saline (SAL) and administered in a volume of 2 ml/kg via intraperitoneal (IP) injection at 0, 2.5, 5, or 7.5 mg/kg.

Experimental design

An initial experiment was conducted to examine whether the FLX dose/regimen selected would induce an antidepressant-like behavioral effect in adolescent female mice. To do this, PD35 female mice were exposed to FLX in their drinking water for 15 consecutive days (PD35-49). Twenty-four h post FLX exposure (PD50), the adolescent mice were tested on the tail suspension test. Based on the results of this experiment (i.e., decreased immobility; Fig. 2C), separate groups of PD35 female mice were randomly selected to receive FLX for the same number of days (PD35-49). However, in this case, the female mice were left undisturbed in their home-cage, for 21 days, post antidepressant exposure. At PD70 (i.e., adulthood) the mice were tested on behavioral responses to cocaine using the conditioned place preference paradigm (Fig. 1A), or their sensitivity to a natural reward (sucrose preference test). Next, to examine whether the altered responses to both cocaine and sucrose observed in adulthood (Fig. 3) were the result of the age of FLX exposure (adolescence vs. adulthood), we conducted a separate set of similar experiments using adult (PD70+) female mice (matched for FLX treatment and behavioral testing time; Fig. 1B). Briefly, as with the adolescent mice, we first examined if the same FLX regimen (250 mg/l; PD70-84) would also mediate and antidepressant-like effect (decrease total

immobility; Fig. 4C) in the tail suspension test, 24 h post antidepressant exposure (i.e., PD85). Also, we evaluated whether such treatment altered preference for cocaine or sucrose, 21 days post antidepressant exposure (i.e., PD105+; see Fig. 5). Separate groups of animals were used across all experiments in order to avoid potential testing carryover effects (see Table 1). A video tracking system (EthovisionXT; Noldus, Leesburg, VA) was used to record the behavioral data, except the tail suspension test, which was scored by observers unaware of antidepressant treatment conditions.

Tail suspension test

The tail suspension test (TST) is commonly used as a measure of behavioral despair – in which mice are placed in an inescapable situation. Specifically, they are suspended by their tail during a single trial of 6 minutes. Initially, mice engage in escape-directed activity, such as body jerks and torsions, but eventually adopt a posture of immobility. It is well accepted across the literature that increases in immobility are indicative of depressive-like behavior (Cryan, Mombereau, & Vassout, 2005). Specifically, because immobility is representative of the animal not being able to successfully cope with the stressful situation,, thus, it parallels the psychological notion of entrapment (despair) that is characteristic in depressed individuals (Lucki, 2001). Interestingly, when animals are acutely administered with drugs that are classified as "antidepressants," before they undergo TST stress, these mice will engage in higher escape-directed behaviors (i.e., assume a posture of immobility less frequently than their vehicle-treated counterparts). As such, decreases in immobility, and increases in escape-directed behavior are correlated with human antidepressant-drug efficacy (Cryan et al., 2005). Since the TST has been pharmacologically validated, by reducing immobility as a function of antidepressant drug exposure, it is more appropriate to describe this paradigm as a test of "antidepressant-like

efficacy," and not as an animal model of "depression" (O'Leary & Cryan, 2009). In our experiment, the total time (s) spent immobile during the last 5 min of the test was the dependent variable.

Conditioned place preference

The conditioned place preference paradigm (CPP) is a commonly used preclinical approach that is based in Pavlovian (or Classical) conditioning principles that allow us to assess the rewarding, or aversive, properties of a given drug (Bardo & Bevins, 2000). In general, the goal of this model is to induce an association between the positive valance of a drug (i.e. cocaine) with the environmental cues of a given compartment in a testing box, as well as the absence of a drug (i.e. drug vehicle) with the opposite compartment. On test day, if the animal spends more time in the compartment where it received the drug, this is considered conditioning. On the other hand, if the animal spends more time in the vehicle-paired side, this is indicative of aversion (Prus, James, & Rosecrans, 2009). There are two different approaches when conducting CPP: biased or unbiased. In a biased design, the natural preference of each of the subjects is considered before conditioning takes place. In this regard, the environment that the animal prefers the least is paired with a drug. Conversely, in an unbiased approach, the researcher decides what compartment to pair with the drug in each individual case (Prus et al., 2009).

In our experiment, we conducted CPP as previously described (Iñiguez et al., 2010b), using a three-compartment apparatus (Alcantara, Warren, Parise, Iñiguez, & Bolaños-Guzman, 2014). The compartments differed in floor texture, as well as wall coloring and pattern. On the preconditioning day (Day 1), mice had free access to explore the entire apparatus for 25 min in order to obtain baseline preference to any of the three compartments (side compartments: 23 × 16 × 36 cm; middle compartment: 9 × 16 × 36 cm, L × W × H). Conditioning trials (25 min, two

per day) were given on four consecutive days (Days 2-5). During the conditioning trials, mice received a SAL injection (1 ml/kg, IP) and were confined to the preferred compartment of the apparatus (biased procedure, see Bardo and Bevins, 2000). After 3 h, mice received cocaine (0, 2.5, 5, or 7.5 mg/kg, IP) and were confined to the opposite (non-preferred) side compartment. Doses of cocaine were selected based on prior work (Hilderbrand & Lasek, 2014). On test day (preference, Day 6), mice were again allowed to freely explore the entire apparatus for 25 min (i.e., PD75 for mice that received FLX-pretreatment during adolescence, and PD110 for mice that received FLX-pretreatment as adults). Data was calculated as a preference score by subtracting the time (sec) spent in the cocaine-paired side during test day (Day 6) from the time spent on the same compartment during the preconditioning day (Day 1). Thus, a positive number indicates higher preference for the cocaine-paired side, whereas a negative number would indicate avoidance of the cocaine-paired side.

Sucrose preference

The sucrose preference test is a paradigm that is commonly used to assess alterations in sensitivity to natural reward in rodents. This test takes advantage of the animal's natural preference for a sweet solution when compared to water only (Eagle, Mazei-Robison, & Robison, 2016). Previous research has shown that sucrose preference is reduced by different chronic stressors, and that this effect is reversed by exposure to antidepressant drugs, like FLX (Liu et al., 2015). Importantly, the sucrose preference test, along with other models such as the intracranial self-stimulation paradigm, has been commonly used to evaluate fundamental features of affect, particularly of anhedonia – the reduced ability to experience pleasure (Vogel, Neill, Hagler, & Kors, 1990; Willner et al., 1987). For this experiment, our approach consisted of a 2-bottle procedure in which mice were given the choice between consuming water or a 1% sucrose

solution. Mice were habituated to drink water from two separate bottles 21-days post FLX exposure (PD70-74 for the adolescent pretreated group, and PD105-109 for the adult pretreated group). Twenty-four h later, one of the bottles was replaced with a 1% sucrose solution, while the other bottle contained water (PD75 for the adolescent antidepressant pretreated group, and PD110 for the adult antidepressant pretreated group). The position of the sucrose bottle was counterbalanced (left vs. right) across the different cages to control for potential side-preference bias. Preference for sucrose over water (sucrose/[sucrose + water]) was used as a measure for sensitivity to reward (Warren et al., 2011).

Design and Data Analysis

Data was analyzed using ANOVA techniques, with FLX pre-treatment (between measure), days of FLX exposure (repeated measure), and cocaine post-treatment (between measure) as sources of variance. Separate analyses were performed between adolescent and adult groups to avoid age-specific influences on locomotor activity. Tukey post hoc tests were used to examine all pairwise comparisons. Planned comparisons were also conducted to examine the hypothesis that FLX pretreatment will alter cocaine-induced reward. Two-tailed Student's t-tests were used for analyses implicating two-group comparisons. Statistical significance was defined as $p<0.05$. Data are presented as mean ± SEM

Chapter 3: Results

FLX Decreases Body Weight in Adolescent Female Mice

Figure 2A shows the effects of adolescent antidepressant exposure (PD35-49) on body weight (g). A mixed-design repeated measures ANOVA showed that weight was influenced by a main effect of FLX treatment (between measure: $F_{(1,123)} = 10.39$, $p<0.05$), a main effect of day of antidepressant exposure (repeated measure: $F_{(14,1722)}=370.88$, $p<0.05$), as well as their interaction (FLX by day of exposure; $F_{(14,1722)}=5.34$, $p<0.05$). Post hoc analyses revealed that when compared to controls (n=63), FLX-exposed mice (n=62) displayed lower body-weight as of the second day of treatment, remaining lower throughout FLX exposure ($p<0.05$, respectively). No enduring differences in body weight, as a function of FLX pre-exposure, were observed at PD70 (i.e., prior to behavioral testing in adulthood, $p>0.05$).

Adolescent FLX Exposure Decreases Immobility in the Tail Suspension Test

Figure 2B-C displays the effects of FLX (PD35-49) on the adolescent tail suspension test. Twenty-four h after antidepressant exposure (PD50), adolescent female mice exposed to FLX displayed a significantly greater latency (sec) to become immobile when compared to the VEH group ($t_{18}=5.0$, $p<0.05$; n=10 per group; Fig. 2B). The FLX treated mice also displayed a lower time immobile ($t_{18}=6.1$, $p<0.05$), when compared to VEH-controls (Fig. 2C), during the last 5-min of the test. Together, this indicates that the FLX dose/regimen selected, mediates a traditional antidepressant-like effect in adolescent female C57BL/6 mice.

Adolescent FLX Exposure Decreases Cocaine Preference in Adulthood

Figure 3A shows the lasting effects of adolescent FLX exposure (PD35-49) on cocaine (0, 2.5, 5, or 7.5 mg/kg) CPP in adulthood (PD70+; N=81). Time spent in the cocaine-paired side varied as a function of adolescent FLX exposure (pretreatment main effect: $F_{(1,73)}=11.48$,

$p<0.05$), as well as cocaine exposure in adulthood (post-treatment main effect: $F_{(3,73)}=25.69$, $p<0.05$). Neither VEH- nor FLX-pretreatment (n=10 per group) resulted in preference for any of the compartments when mice were conditioned to saline ($p>0.05$). In contrast, we found that VEH-pretreated mice conditioned to 2.5 (n=11), 5 (n=10), or 7.5 mg/kg (n=10) cocaine, displayed reliable conditioning, when compared to VEH-pretreated/saline-conditioned mice ($^{\alpha}p<0.05$). Planned comparisons indicated that FLX pretreatment also mediated reliable conditioning to the compartment paired with 2.5 (n=11), 5 (n=9), and 7.5 (n=10) mg/kg cocaine, when compared to FLX-pretreated/saline-conditioned mice ($p<0.05$, respectively). Interestingly, the FLX-pretreated female mice conditioned to 5 and 7.5 mg/kg cocaine spent significantly less time in the drug-paired compartment when compared to VEH-pretreated mice receiving the same doses of cocaine in adulthood ($*p<0.05$, respectively). No differences in distance traveled (cm), as a function of adolescent FLX pretreatment, were observed during the preconditioning phase (PD70, $p>0.05$; data not shown) – indicating no differences in general locomotor activity between the groups.

Adolescent FLX Exposure Decreases Sucrose Preference in Adulthood

Figure 3B-C shows the lasting effects of adolescent FLX exposure (PD35-49) on sucrose preference in adulthood (PD70+). A student's t test indicated that adult female mice exposed to FLX during adolescence (n=12) displayed a decrease in preference for a 1% sucrose solution when compared to VEH-pretreated (n=12) controls ($t_{22}=12.89$, $p<0.05$; Fig. 2B). No differences in total liquid intake (water + sucrose) were observed between the groups ($p>0.05$; Fig. 2C).

FLX Decreases Body Weight in Adult Female Mice

Figure 4A shows the effects of adult antidepressant exposure (PD70-84) on body weight (g). A mixed-design repeated measures ANOVA showed that weight was influenced by a main

effect of FLX (between measure: $F_{(1,122)}$=10.17, p<0.05), a day main effect of antidepressant exposure (repeated measure: $F_{(14,1708)}$=68.28, p<0.05), as well as their interaction (FLX by day of exposure; $F_{(14,1708)}$=28.74, p<0.05). Post hoc analyses revealed that when compared to controls (n=62), adult mice exposed to FLX (n=62) displayed lower body weight the first 8 days of treatment (PD71-PD78, p<0.05, respectively). No differences in body weight were observed the last 6 days of FLX exposure (PD79-84; p>0.05). Similarly, no enduring differences in body weight, as a function of FLX pre-exposure, were observed later in life (PD105), prior to behavioral testing (see Fig. 4A inset).

Adult FLX Exposure Reduces Immobility in the Tail Suspension Test

Figure 4B-C displays the effects of FLX (PD70-84) on the adult tail suspension test. Twenty-four h after antidepressant exposure (PD85), adult female mice exposed to FLX (n=10) displayed a significantly greater latency (sec) to become immobile (t_{18}=6.84, p<0.05), when compared to their VEH treated counterparts (n=10; Fig. 4B). The FLX treated mice also displayed a lower time immobile (t_{18}=3.37, p<0.05), when compared to VEH-controls (Fig. 4C), during the last 5-min of the test. Together, this indicates that the FLX dose/regimen selected, mediates a traditional antidepressant-like effect in adult female C57BL/6 mice.

Adult FLX Exposure Reduces Cocaine Preference Later in Life

Figure 5A shows the enduring effects of adult FLX exposure (PD70-84) on cocaine CPP (N=80). Here, the time spent in the cocaine-paired side varied as a function of adult FLX exposure (pretreatment main effect: $F_{(1,72)}$ =7.63, p<0.05), as well as cocaine exposure later in life (post-treatment main effect: $F_{(3,72)}$=8.72, p<0.05). Neither the VEH-pretreated (n=10) nor the FLX-pretreated (n=10) animals displayed a preference for any of the compartments when they were conditioned to saline (p>0.05). In contrast, preplanned comparisons indicated that female

mice pre-exposed with VEH (PD70-84) conditioned to 2.5 (n=10), 5 (n = 10), and 7.5 mg/kg (n=10) cocaine showed reliable conditioning when compared to VEH-pretreated/saline-conditioned mice (p<0.05, respectively). Similarly, the FLX-pretreated mice conditioned to 7.5 (n=10), but not 2.5 (n=10) or 5 (n=10), mg/kg cocaine showed reliable conditioning when compared to FLX-pretreated/saline-conditioned mice (p<0.05). Interestingly, FLX-pretreated mice conditioned to 5 and 7.5 mg/kg cocaine spent significantly less time in the drug-paired compartment when compared to VEH-pretreated mice receiving the same doses of cocaine (p<0.05, respectively). No differences in total distance traveled (cm) were noted between the FLX-pretreated animals and their respective VEH-pretreated controls during the preconditioning phase (PD105; Data not shown).

Adult FLX Exposure Decreases Sucrose Preference Later in Life

Figure 5B-C shows the effects of adult FLX exposure (PD70-84) on sucrose preference, 21-days post antidepressant exposure (PD105; n=12 per group). A student's t test indicated that FLX pre-exposed mice displayed a decrease in preference for a 1% sucrose solution when compared to VEH-pretreated (n=12) controls (t_{22}=12.89, p<0.05; Fig. 5B). No differences in total liquid intake (water + sucrose) were observed between the groups (p>0.05; Fig. 5C).

Chapter 4: Discussion

SSRI's, like FLX, are often prescribed to the adolescent female population for the treatment of numerous illnesses, although, primarily for the management of MDD (John et al., 2016; Schroder et al., 2017; Steiner et al., 1995). This is surprising given that pharmacodynamic differences between developing and adult organisms are commonly reported as a function of psychotropic drug exposure (Correll, Kratochvil, & March, 2011; Iñiguez et al., 2008), and that such treatments mediate long-lasting neurobehavioral alterations (Brooks, O'Donnell, & Frost, 2016; Carlezon et al., 2003; Olivier et al., 2011). Another problem is that animal studies examining for potential enduring side effects, as a result of early-life psychotropic exposure, have largely excluded females as subjects. Recent data, in male rodents, indicate that juvenile exposure to FLX alters responses to cocaine in adulthood (Iñiguez et al., 2015), potentially altering drug abuse vulnerability. However, whether adolescent FLX exposure results in prolonged alterations in sensitivity to reward-related stimuli, in females specifically, has not been evaluated. As such, the purpose of the present investigation was to examine if changes in responses to cocaine and sucrose would be observed in adult female mice (PD70+) pretreated with FLX during adolescence (PD35-49).

To do this, we first evaluated whether the FLX dose/regimen selected (250 mg/kg in drinking water for 15 days) would mediate an antidepressant-like effect in juvenile (PD35-49) and adult (PD70-84) female mice. Specifically, we evaluated whether or not FLX would decrease immobility in the tail suspension test, a behavioral measure of despair that is widely used to evaluate antidepressant-like efficacy across the literature (Cryan et al., 2005). Not surprisingly, independent of age of antidepressant exposure, we found that SSRI treatment increased escape directed behaviors (i.e., increase in the time to become immobile, along with

decreased total immobility; Figs. 2B-C and 4B-C) – a traditional antidepressant-like effect. Furthermore, adolescent and adult FLX exposure resulted in decreases in body weight-gain across days of treatment (Figs. 2A and 4A), similar to what others have previously reported (Amodeo et al., 2015). However, the decreases in body weight were not observed 21-days post treatment in either age group, thus, suggesting that FLX history does not result in enduring decreases of body weight in female C57BL/6 mice.

Adolescent FLX exposure decreased sensitivity to both drug and natural- reward related stimuli in adulthood. Specifically, in the cocaine place conditioning experiments (Fig. 3A), FLX-pretreated mice displayed lower preference scores for cocaine at the 5 and 7.5 mg/kg doses, when compared to respective saline-pretreated controls exposed to the same cocaine regimen. Importantly, no differences in general locomotor activity were noted between the groups on the preconditioning day, thus, uncovering a decrease in the reward incentive of cocaine (Bardo & Bevins, 2000). To further explore whether this decrease in preference for cocaine would generalize to a natural reward, we examined the effects of adolescent antidepressant exposure on the 2-bottle sucrose choice test in adulthood (Fig. 3B-C). Here, we found that FLX-pretreated animals displayed a decrease in preference for a 1% sucrose solution, without altering total liquid intake – a response that is commonly described as an anhedonia-like phenotype (Willner, Towell, Sampson, Sophokleous, & Muscat, 1987). Interestingly, this behavioral profile is in direct contrast with previous work conducted in male rodents, which display an enduring increase in preference to both cocaine (Iñiguez et al., 2015) and sucrose (Iñiguez et al., 2010a), as a function of adolescent FLX treatment. This is an important finding, as it demonstrates how juvenile SSRI exposure mediates differential effects on reward sensitivity as a function of sex in adulthood.

To evaluate whether the decreased sensitivity to reward-related stimuli was dependent on the age of FLX exposure (i.e., adolescence), we followed with a series of similar experiments using adult (PD70) female mice (see Fig. 1B), as a positive control group for age of SSRI exposure. Unexpectedly, adult antidepressant pretreatment (PD70-84) resulted in a similar behavioral response when these animals were tested for cocaine preference, 21-days post FLX pre-treatment (Fig. 5A). Specifically, the adult FLX-pretreated mice conditioned to 5 and 7.5 mg/kg cocaine, displayed lower preference scores when compared to the VEH-pretreated animals exposed to the same doses of the stimulant. Analogously, when assessing sensitivity to sucrose, we found also that FLX pre-exposure decreased preference for the 1% sucrose solution later in life (Fig. 5B-C). Collectively, our data are consistent with previous work conducted in female rodents indicating that neonatal (PD5-18) exposure to SSRI's causes enduring depression-related behaviors, as per reductions in the rewarding properties of sucrose (Popa, Lena, Alexandre, & Adrien, 2008). Yet, here, we extend these findings to the adolescent (PD35-49) and adult (PD70-84) stages of development.

The clinical implications of our findings are challenging to interpret. For example, reductions in cocaine and sucrose preference may help explain why clinical data suggests that SSRI's may reduce the risk of substance abuse, particularly for cocaine (Moeller et al., 2007; Oliveto et al., 2012; Walsh, Preston, Sullivan, Fromme, & Bigelow, 1994), implying that if cocaine and sucrose are less rewarding, this phenotype may be indicative of reduced drug abuse potential. However, a different interpretation of this reward devaluation may be indicative of a prolonged anhedonia-like behavioral profile after antidepressant exposure (Popa et al., 2008). Supporting this theory, recent clinical findings suggest that SSRI discontinuation leads to prolonged decreases in pleasurable stimuli, such as sexual performance (Reisman, 2017) – a

prospective additional endophenotype of depressive-like behavior (Neill, Vogel, Hagler, Kors, & Hennessey, 1990), that may potentially be more specific to the female population (Khazaie, Rezaie, Rezaie Payam, & Najafi, 2015).

The neurobiological mechanisms underlining this lowered reward behavioral phenotype are currently not known. Because behavioral assessment was conducted 21-days after FLX exposure, we argue that the decreases in sensitivity to cocaine and sucrose are the result of enduring neuroplastic changes. For example, in male rodents, adolescent FLX exposure results in long-term molecular signaling alterations within discreet mood-related brain regions, such as the ventral tegmental area (Iñiguez et al., 2014), the amygdala (Homberg et al., 2011), the frontal cortex (Wegerer et al., 1999), and the hippocampal formation (Klomp, Vaclavu, Meerhoff, Reneman, & Lucassen, 2014). Interestingly, in adult female rats, chronic exposure to FLX alters hippocampal neurogenesis one month after antidepressant exposure (Airan et al., 2007), potentially mediating, at least in part, the lowered sensitivity to reward-related behavior observed in the present investigation. Of course, future work will be needed to examine how enduring FLX-induced alterations in hippocampal neuroplasticity may influence sensitivity to reward-related stimuli, given that this brain region is implicated in mediating preference for cocaine in the place-conditioning paradigm (Meyers, Zavala, Speer, & Neisewander, 2006; Nygard et al., 2013).

A limitation of the present investigation is that we did not control for estrous cyclicity in our experimental animals. In females and males, estradiol has been proposed to be a modulator of reinforcing stimuli, including drugs of abuse like cocaine (Kerstetter et al., 2012; Kerstetter & Kippin, 2011). As such, future studies will be necessary to delineate the potential role that sex steroids may play in the behavioral responses observed, as a function of FLX pre-exposure.

Another limitation of our work is that we are evaluating alterations in reward three-weeks post FLX exposure in normal animals. However, given that FLX is prescribed to the female population for numerous illnesses in addition to MDD, such as anxiety, premenstrual dysphoric disorder, and pain (Mika, Zychowska, Makuch, Rojewska, & Przewlocka, 2013; Steiner et al., 1995), we believe that this is an appropriate approach to initially assess for enduring side effects as a function of antidepressant exposure.

Conclusion

The high prescription rate of SSRI's for the management of mood-related illnesses in the female population is undeniable (Schroder et al., 2017). Yet, most preclinical studies examining for potential enduring antidepressant-induced consequences have mostly included male animals, so the possibility of sex differences in the outcome of drug-induced side effects, later in life, has been largely ignored. Here, we report that exposure to FLX, in adolescent and adult female C57BL/6 mice, decreases cocaine and sucrose preference, 21-days post antidepressant exposure – a behavioral profile indicative of an anhedonia-like phenotype. In light of this, future work will be necessary to delineate the precise neurobiological mechanisms by which FLX history decreases sensitivity to cocaine and sucrose, in females specifically, in an age independent manner.

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Table 1. Experimental groups.

Group	Drug	n	Age	Time	Procedure	Data
1	CON	10	PD35-49	24 h	Tail Suspension Test (PD50)	Fig. 2B-C
	FLX	10	PD35-49			
2	CON	41	PD35-49	21 d	Cocaine Place Preference (PD70-75)	Fig. 3A
	FLX	40	PD35-49			
3	CON	12	PD35-49	21 d	Sucrose Preference (PD70-75)	Fig. 3B-C
	FLX	12	PD35-49			
4	CON	10	PD70-84	24 h	Tail Suspension Test (PD85)	Fig. 4B-C
	FLX	10	PD70-84			
5	CON	18	PD70-84	21 d	Cocaine Place Preference (PD105-110)	Fig. 5A
	FLX	18	PD70-84			
6	CON	12	PD70-84	21 d	Sucrose Preference (PD105-110)	Fig. 5B-C
	FLX	12	PD70-84			

CON, control; d, day; FLX, fluoxetine; h, hour; PD, postnatal day.

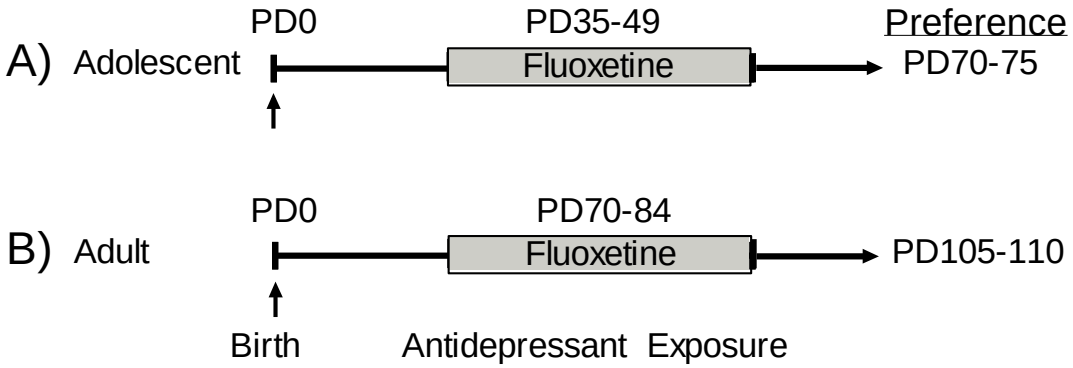

Figure 1: Experimental Design. Separate groups of adolescent (postnatal day [PD]-35) and adult (PD70) female C57BL/6 mice received vehicle (control) or fluoxetine (FLX; 250 mg/ml, in their drinking water) for 15 consecutive days. Twenty-one days later, mice were tested on their preference for cocaine (0, 2.5, 5, or 7.5 mg/kg) using the place conditioning test, or sucrose (1%) preference using the 2-bottle choice test. Specifically, (**A**) adolescent FLX pretreated animals initiated behavioral testing at PD70, while (**B**) adult FLX pre-exposed mice initiated behavioral testing at PD105

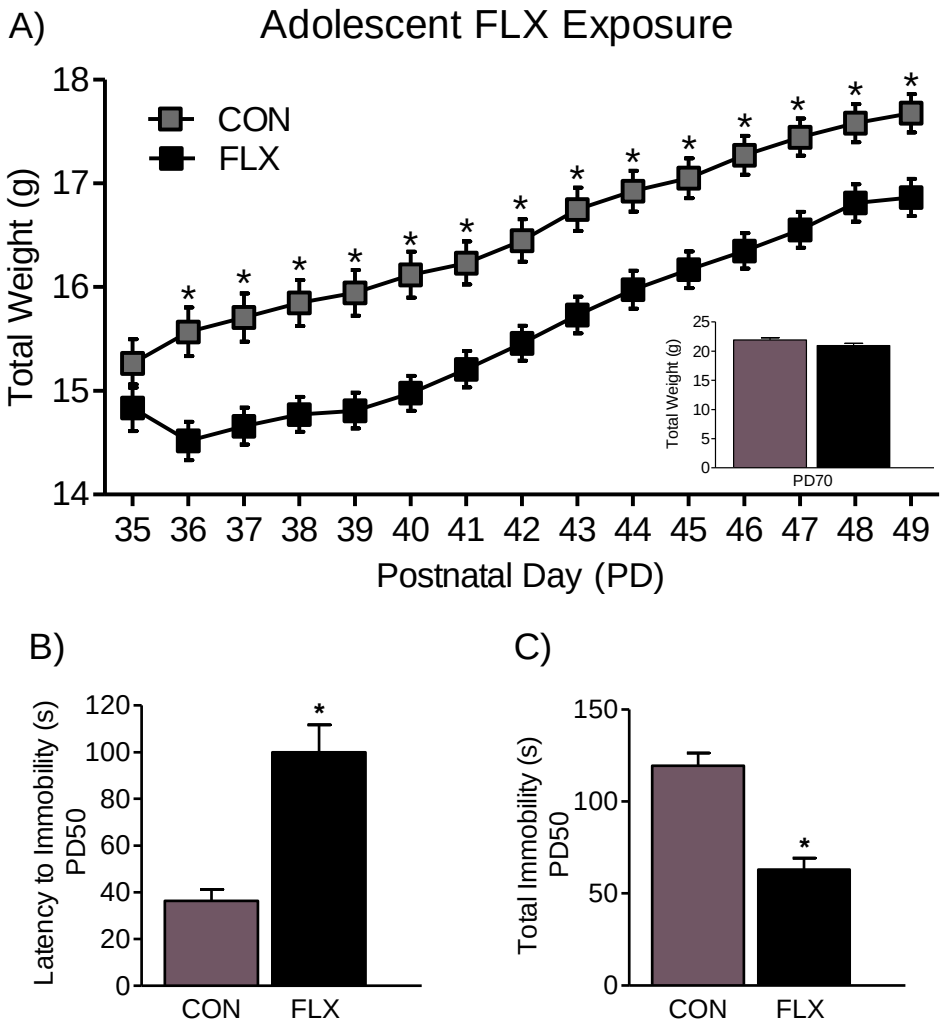

Figure 2: Effects of adolescent fluoxetine (FLX) exposure on body weight gain and the tail suspension test. (**A**) Body weight increased across days of treatment (postnatal day [PD] 35-49), regardless of FLX or water-control (CON) conditions. However, when compared to CON's, FLX-exposed mice displayed lower weight gain as of the second day (PD36) of antidepressant exposure ($p<0.05$). Twenty-one days later (PD70), no differences in body weight were noted between the groups ($p>0.05$; inset). Data are presented as average weight across days and antidepressant exposure (mean ± SEM, in grams). (**B**) Twenty-four h post antidepressant exposure (PD50), a separate group of adolescent mice was tested in the tail suspension test. FLX-exposed mice displayed higher time (s) to adopt a posture of immobility, (**C**) as well as a lower time spent immobile, when compared to CON's ($p<0.05$). Data are presented as total time in seconds (mean ± SEM). *Significantly different when compared to CON ($p<0.05$).

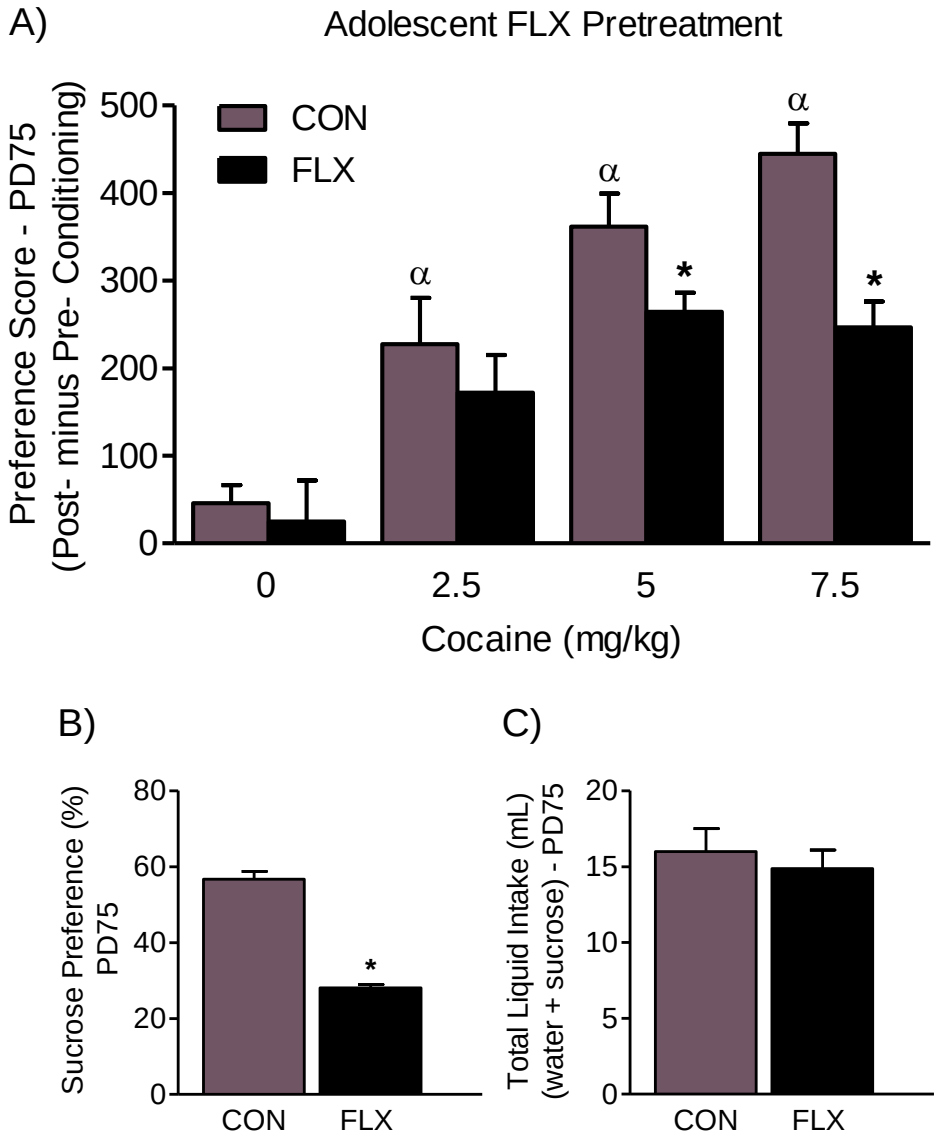

Figure 3. Effects of adolescent fluoxetine (FLX) exposure on reward-related behavior in adulthood. (**A**) Three-weeks after adolescent antidepressant exposure (postnatal day [PD]-70+), FLX-pretreated mice displayed decreased sensitivity to 5 and 7.5 mg/kg cocaine, when compared to water-pretreated (CON) mice receiving the same doses of cocaine (n=9–11 per experimental group; $p<0.05$). *Within cocaine-dose group comparison ($p<0.05$). $^{\alpha}$Significantly different when compared to age-matched controls conditioned to saline ($p<0.05$). (**B**) Adolescent FLX pretreatment reduced preference for a 1% sucrose solution 3-weeks after antidepressant exposure (n=12 per group; $p<0.05$). (**C**) No differences in total liquid intake were observed between the experimental groups ($p>0.05$).

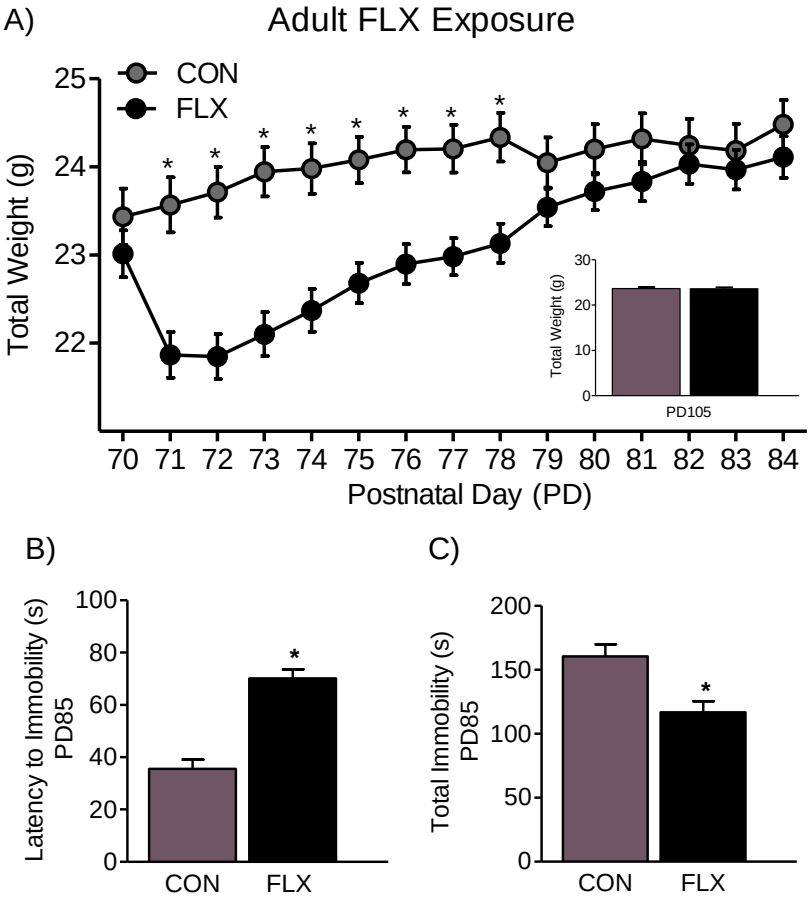

Figure 4: Effects of adult fluoxetine (FLX) exposure on body weight gain and the tail suspension test. (**A**) Body weight increased across days of treatment (postnatal day [PD] 70-84), regardless of FLX or water-control (CON) conditions. However, when compared to CON's, FLX-exposed mice displayed lower weight gain as of the second day of antidepressant exposure (PD71), remaining lower until PD78 ($p<0.05$). Twenty-one days post FLX exposure (PD105) no differences in body weight were noted between the groups ($p>0.05$; inset). Data are presented as average weight across days and antidepressant exposure (mean ± SEM, in grams). (**B**) Twenty-four h post antidepressant exposure (PD85), a separate group of adult mice was tested in the tail suspension test. FLX-exposed mice displayed higher time (s) to adopt a posture of immobility, (**C**) as well as a lower time spent immobile, when compared to CON's ($p<0.05$). Data are presented as total time in seconds (mean ± SEM). *Significantly different when compared to CON ($p<0.05$).

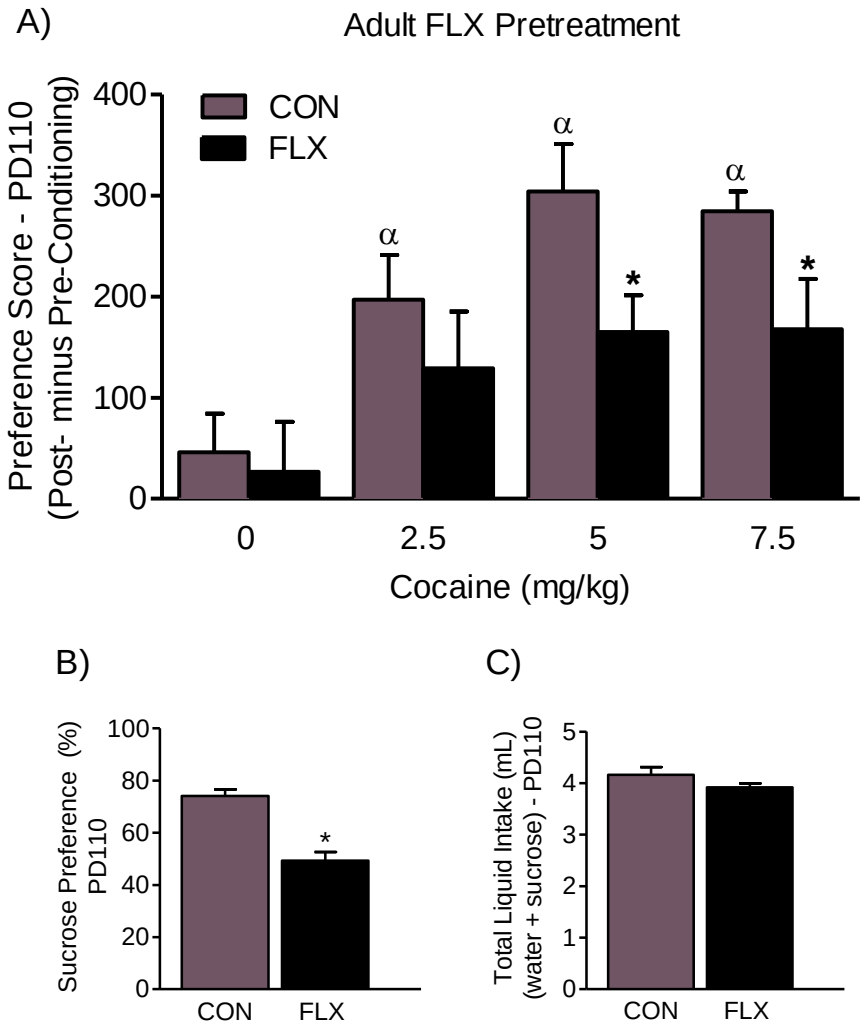

Figure 5: Enduring effects of adult fluoxetine (FLX) exposure on reward-related behavior. (**A**) Three-weeks after adult antidepressant exposure, FLX-pretreated mice displayed decreased sensitivity to 5 and 7.5 mg/kg cocaine, when compared to water-pretreated controls (CON) mice exposed to the same doses of cocaine (n=10 per experimental group; $p<0.05$). *Within cocaine group comparison ($p<0.05$). $^{\alpha}$Significantly different when compared to age-matched controls conditioned to saline ($p<0.05$). (**B**) Adult FLX pretreatment reduced preference for a 1% sucrose solution 3-weeks after antidepressant exposure (n=12 per group; $p<0.05$). (**C**) No differences in total liquid intake were observed between the experimental groups ($p>0.05$).

Vita

Francisco J. Flores Ramirez was born to Jorge Flores Nieto and Elva Ramirez Zamarripa in La Piedad, Michoacán, México. He graduated from Colton High School in Colton, California in June 2007 and enrolled in San Bernardino Valley College (SBVC) in San Bernardino, California, the following semester. After three years in community college, Francisco enrolled into California State University San Bernardino, where he obtained his Bachelors of Arts degree in Psychology in December of 2013. He became interested in Neuroscience and began research work with Dr. Sergio D. Iñiguez, who studies the neurobiological factors that underlie mood related disorders. He entered the Social, Cognitive, and Neuroscience program at UTEP in August, 2016, where he continues to receive mentorship from Dr. Sergio D. Iñiguez to investigate the short- and long-term effects of exposure to psychotropic medications using female mice as a model system. Francisco has presented his research work in over 15 conferences related to neuroscience. He is co-author on one published article in *Neurobiology of Stress* and one published article in *Biological Psychiatry*.

Contact Information: fjfloresram@miners.utep.edu

Permanent address: 125 Vaquero Ln. Apt. 137

El Paso, TX, 79912

This manuscript was typed by Francisco J. Flores Ramirez