

ENVIRONMENTAL ENRICHMENT AND SEROTONERGIC ALTERATIONS ON  
DEPRESSIVE-LIKE STATES IN RATS

by

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## **Abstract**

Individuals suffering from depression primarily rely on pharmacological interventions to alleviate the incapacitating symptoms of the disorder. In addition to genetic differences underlying the etiology of depression, environmental factors play a key role as well. For example, environmental enrichment results in various neurotransmitter alterations, significantly affecting serotonin. To test the efficacy of novel antidepressant drugs in the preclinical laboratory setting, researchers commonly implement the forced swim test (FST) for rats or mice. However, the effect of environmental enrichment on the expression of depressive-like states in the FST is unclear, and it is unknown whether environmental enrichment or social isolation can alter the efficacy of the commonly prescribed antidepressant drug, fluoxetine. In the present study, locomotor activity and FST performance were measured after 30 days of rearing in enriched (EC), standard (SC), and isolated (IC) conditions. Results showed that regardless of the significant effect of fluoxetine on locomotor activity in EC, SC, and IC rats, fluoxetine failed to increase swimming and decrease immobility in all three environmental conditions, with enriched-fluoxetine rats displaying significantly less swimming behavior in the FST than enriched rats receiving vehicle control injections. These results suggest that differential rearing, specifically environmental enrichment, can alter the efficacy of antidepressants and may suggest that enrichment reverses the effects of fluoxetine.

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# **CHAPTER 1 - Introduction**

## **Aims**

Mood disorders, such as depression, can detrimentally affect people's lives both mentally and physically. Of all people affected by major depression, approximately 4% eventually commit suicide (Barlow, 2005). With mood disorders affecting millions of people worldwide, depression has undoubtedly become a major global health burden (Licinio & Wong, 2005; Murray & Lopez, 1997), and there are many factors that lead to the etiology of depression. Both the environment and the neurotransmitter serotonin are known to play key roles in the development of this disorder.

The current study determined if an enriched rearing environment during development could attenuate the expression of depressive-like states. In addition, the current study examined the effects of the selective serotonin reuptake inhibitor (SSRI) fluoxetine (20 mg/kg) on the development of depressive-like states in enriched, isolated, and standard-housed rats. The findings from these experiments aid in our understanding of the serotonergic alterations that may occur throughout the rearing process, and how these alterations can affect the function of SSRIs.

The general research objective of the current study was to understand how the combination of different rearing environments and serotonergic alterations via the SSRI fluoxetine could affect depressive-like states in male rats.

## **Rodent Models of Depression**

Interestingly, many available antidepressant drugs reduce general locomotor behavior in rats and increase escape-directed behavior in the forced swim test (FST), a common method of measuring depression or behavioral despair in rats. An increase of escape-directed behavior is



believed to reflect an antidepressant-like state (Slattery & Cryan, 2012). The neural substrates involved in the drug-induced decrease in locomotor behavior in an open-field test and the increase in escape-directed behavior in the FST are important for the accurate ascription of antidepressant drugs. For example, if a drug is investigated for having antidepressant-like properties (decreased immobility in the FST), but the drug increases locomotor behavior in the open-field test, then it cannot be concluded that the drug is a viable pharmacologic option to treat depression. This erroneous conclusion would lead to Type I errors, or false-positives.

In addition to preventing false-positives or false-negatives in the forced swim test, the open field test (OFT) has been used extensively to study the general locomotor differences induced by differential rearing, with enriched condition (EC) rats generally showing a decreased response (i.e., less locomotion) to a novel environment (Bardo et al., 1995; Cain et al., 2006).

Contrary to the extensively studied open-field test, relatively few studies to date have examined the effects of environmental enrichment on the alteration of depressive-like behavior in the rodent FST. The forced swim test was first proposed by Porsolt and colleagues (1977) and has developed into the most commonly used animal model for assessing antidepressant-like behavior. This is due to several factors, including its specificity, predictive validity, inter-laboratory reliability, and ease of use (Slattery & Cryan, 2012). As of June, 2014, a PubMed.gov search of "forced swim test" resulted in more than 3,800 peer-reviewed publications, providing evidence that the forced swim test has become and continues to be a reliable experimental approach to assess the efficacy of antidepressants with high predictive validity. The forced swim test typically involves two sessions, a pretest and a test session, followed by the scoring of active (swimming and climbing) or passive (immobility) behavior when rodents are forced to swim in a cylinder of water in which escape is not possible (Slattery & Cryan, 2012). The pretest is

implemented to facilitate immobility, or behavioral despair, in the test session. As a result, rats in the forced swim test that display more active, escape-directed behavior and reduced immobility are presumably displaying a decreased amount of helplessness and despair, and can be operationalized as less depressed.

A controversial argument and general concern about the FST is that it does not measure a depressive-like state at all. Rather, critics of the FST argue that rats are learning to become immobile during the test swim because they realize (based on their pretest experience in the first session) that they will soon be removed from the inescapable environment by the experimenter. Therefore, it is presumed that rats should develop a passive coping strategy to avoid unnecessarily spending energy in their futile efforts to escape the cylinder. This idea of learned immobility has been debated (De Pablo et al., 1989; Jefferys & Funder, 1994; West, 1990), but is seemingly an inaccurate assumption. The learned immobility theory oversimplifies the FST situation from an anthropomorphic perspective (Cryan et al., 2005). Technically speaking, a rat opting for the strategy of infinite immobility would inevitably lead to drowning, whereas if the rat strategically displays escape-directed behavior, it may end up expediting the drowning process but would yield a minute chance of survival. This difficult decision of strategic choice is shown in earlier studies with rats exposed to prolonged periods of swimming (Richter, 1957).

Furthermore, recent studies have provided quantitative evidence countering the argument for learned immobility. For example, in Wistar-Kyoto rats (a strain used to model depression due to their increased expression of immobility in the FST), there appears to be a negative relationship between increased FST immobility and adrenocorticotropin (ACTH) hormone and corticosterone (CORT) secretion, such that rats displaying prolonged behavioral immobility in the FST also show amplified ACTH and CORT secretion (Dal-Zotto et al., 2000; Rittenhouse et

al., 2002). The learned immobility theory from De Pablo (1989) and West (1990) fails to consider that immobility in the FST remains a stressful behavioral state for the rat, which would not coincide with and would not be alleviated by a learned process.

A final point countering the learned immobility hypothesis is illustrated by the experimental observation that several known antidepressant drugs can reduce immobility in a single session without the need of facilitating immobility via the pretest. This reduced immobility (antidepressant-like state) is evident after both acute (Borsini & Meli, 1988) and chronic (Overstreet et al., 2004) administration of various antidepressant drugs. Taken together, these previous findings suggest that learned immobility is not a factor in the behavioral effects of antidepressant drugs, and that the FST is a valid measure of testing depressive-like states in rats.

### **Fluoxetine Neuronal Mechanisms of Action**

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) that was first developed and reported in 1974 (Wong et al., 1974) and is now effectively used to treat a wide variety of mood disorders, including depression. Fluoxetine works by delaying the reuptake of the neurotransmitter serotonin (5-HT), resulting in more 5-HT residing in the synapse for a longer period of time (Fuller et al., 1974; Tatsumi, 1997). There appear to be at least 14 receptor subtypes that mediate the actions of 5-HT, which can sensitize or desensitize upon chronic SSRI administration (Kriegebaum et al., 2010). The 5-HT<sub>1A</sub> is a serotonin receptor subtype that regulates the firing rate of serotonergic neurons (Schiller et al., 2006) and is likely to change following chronic exposure to fluoxetine (Olivier et al., 2011). The 5-HT<sub>1A</sub> consists of inhibitory autoreceptors of serotonin, and when 5-HT<sub>1A</sub> is chronically activated via repeated fluoxetine exposures, these inhibitory receptors are desensitized, leading to increased serotonin release in terminal regions (Stahl, 1997).

The primary protein responsible for the movement of 5-HT back into the presynaptic membrane is the serotonin transporter (SERT). Studies have shown that administration of a 5-HT<sub>1A</sub> receptor antagonist (WAY 100635) results in increased 5-HT output that potentiates the effects of fluoxetine (10 mg/kg) in the frontal cortex (Hervás & Artigas, 1998). In other words, inhibiting the receptor subtype responsible for the transportation of 5-HT back into the presynaptic membrane can accelerate the effects of antidepressant drugs, such as fluoxetine, that inhibit 5-HT reuptake.

### **Fluoxetine and FST Performance**

Fluoxetine is one of the most commonly prescribed antidepressant drugs, and is actually the only SSRI registered for treatment of the pediatric population (Olivier, 2011). Since it was first reported (Wong et al., 1974), it has been extensively studied in rodent models of depression. Research has shown that the antidepressant effects of fluoxetine, as observed in the FST, are not only dose-dependent, but also differ when administered acutely or chronically.

The dose-dependent effects of fluoxetine in the FST are fairly well-documented. Kulkarni et al. (2007) concluded that the required dose of fluoxetine needed to elicit a significant increase in escape-directed behavior in 50% of mice (ED<sub>50</sub>) was 18 mg/kg, i.p. Nonetheless, significant reductions in immobility have been found using a smaller dose (5-10 mg/kg) as well, even when the smaller doses were administered sub-chronically (Detke et al., 1995; Page et al., 1999; Refaey & Hasan, 2011; Reneric & Lucki, 1998). When administered sub-chronically (23.5 hours, 5 hours, and 1 hour before the FST), it appears that 20 mg/kg, i.p. fluoxetine yields robust effects, and is recommended as a positive control when investigating other antidepressant compounds in the laboratory (Slattery & Cryan, 2012).

In addition to the dose-dependent effects of fluoxetine, its antidepressant efficacy also differs when administered acutely, sub-chronically, or chronically. Generally speaking, several low doses of fluoxetine are ineffective when administered acutely, but effective when administered chronically (usually a 14-day period; Cryan et al., 2005). For example, chronic administration of 1, 2 and 5 mg/kg fluoxetine can significantly reduce depressive-like states, but when the same doses are administered acutely or sub-chronically (usually 3 injections over a 24 hour period), no antidepressant-like effect is found (Detke et al., 1997). Furthermore, it has been shown that fluoxetine (10 mg/kg), when administered chronically, can reduce immobility in isolated (IC) rats to a level that reaches standard condition (SC) values (Brenes et al., 2009).

### **Differential Rearing-Induced Changes in Serotonin**

Research has shown that differentially rearing rats in enriched (EC), isolated (IC), or standard (SC) environments during adolescence leads to both neurochemical and behavioral changes in the brain (Renner & Rosenzweig, 1987; Rosenzweig et al., 1972). This paradigm is typically used to investigate the beneficial effects of an enriched environment compared to an isolated environment. The differential rearing period of 30 days has become a standard rearing duration for rodents that yields profound, reproducible, and robust behavioral changes between EC and IC rats (Green et al., 2010; Renner & Rosenzweig, 1987). The standard condition (SC) is usually not employed to control for all the behavioral differences observed between EC and IC rats, but rather to provide a known laboratory standard for comparison (Cain et al., 2012; Gill et al., 2012; Wooters et al., 2011).

Differentially rearing rats in an enriched environment during the post-weaning period can result in enhanced synaptic plasticity, as evidenced by the enlargement of synaptic boutons, higher density of dendritic spines, increased long term potentiation, and other enhancements in

synaptic transmission (Artola et al., 2006; Green & Greenough, 1986; Renner & Rosenzweig, 1987; Sharp et al., 1985; van Praag et al., 2000). Differentially rearing rats in various environments can lead to altered function of not only synaptic plasticity, but brain-derived neurotrophic factor (BDNF) and hypothalamic-pituitary-adrenal axis (HPA) activity (Simpson & Kelly, 2011) as well.

Several neurotransmitters are also altered during the differential rearing process, including 5-HT (Fone & Porkess, 2008). Serotonin is heavily involved in the development and persistence of several mood disorders, such as depression (Lanni et al., 2009). Furthermore, people suffering from major depression have decreased hippocampal volume. Likewise, rats exposed to chronic stress display similar reductions in hippocampal neurons and display significant dendritic atrophy in related brain regions (Hasler et al., 2004). Following the environmental enrichment period, rats have enhanced expression of the gene for the 5-HT<sub>1A</sub> receptor in the hippocampus (Rasmuson, 1998), suggesting more functional serotonergic regulatory mechanisms in EC rats. These findings also provide support that decreased hippocampal structure and function could serve as a possible endophenotype in animal models of depression.

Of the few studies investigating enrichment and performance in the forced-swim test, enrichment has been found to produce antidepressant-like effects and increased serotonin concentrations in the prefrontal cortex compared to control and isolation-reared rats, which correlate with behavioral performance in the FST (Brenes et al., 2008). Furthermore, differential rearing has been shown to modify forced swimming behavior and hippocampal concentrations of norepinephrine and serotonin (Brenes et al., 2009).

Recent studies suggest enrichment-induced neuronal alterations that mimic SERT inhibition. For example, environmental enrichment with voluntary exercise can lead to the reduction of tryptophan-hydroxylase (TPH)-immunoreactive neurons, and these reductions are similar in magnitude to what is observed in fluoxetine-treated rats (MacGillivray et al., 2012). TPH is one of the enzymes responsible for the synthesis of serotonin (Côté et al., 2003), and is found to be increased in postmortem tissue of treatment-naïve suicide victims (Bach-Mizrachi et al., 2006), which implies that TPH works as a possible stimulatory response to compensate for low 5-HT levels in depression (Klomp et al., 2014). These findings suggest that some of the effects of both enrichment and SSRIs may be acting through common mechanisms, but more research is needed to validate this assumption.

The prefrontal cortex (PFC) appears to play an integral role in the neural circuits involved in depression (Drevets et al., 1992). For instance, many established antidepressant drugs alter PFC activity by increasing dorsal PFC activity and decreasing ventral PFC activity (Ressler & Nemeroff, 2000). Because enriched and isolated rats differ in PFC functionality (Fone & Porkess, 2008), the benefits of enrichment observed in FST behavior could stem from enhanced 5-HT function in the PFC (Brenes et al., 2008; Llorens-Martin et al., 2007).

Coinciding with these findings, rats reared in an isolated environment exhibit altered serotonergic function, with increased 5-HT<sub>1A</sub> receptor binding in the CA fields of the hippocampus (Fone & Porkess, 2008; Preece et al., 2004). However, there are inconsistencies in the literature in regard to isolation-induced 5-HT alteration, particularly involving the 5-HT<sub>1A</sub> receptor. Furthermore, these alterations appear to be region specific. For example, isolation rearing appears to decrease basal 5-HT turnover in the nucleus accumbens compared to standard-housed rats (Heidbreder et al., 2000), but has little to no effect in the prefrontal cortex or caudate

putamen (Jones et al., 1992). Furthermore, Muchimapura and colleagues (2003) found that isolation rearing alters presynaptic 5-HT<sub>1B</sub>, but not post-synaptic dorsal hippocampal 5-HT<sub>1A</sub> receptors.

Although there is an abundance of literature investigating the effects of fluoxetine and other SSRIs on FST behavior, relatively little is known about how these well-established drugs exert their antidepressant-like effects in differentially reared rats. The literature suggests a possible rearing-induced EC and IC divergence in response to various SSRIs. For example, Raz and Berger (2010) concluded that antidepressants, particularly SSRIs, may normalize or stabilize serotonin function and restore the potential behavioral changes produced by isolation rearing. Furthermore, administration of the SSRI sertraline can reduce depressive-like states in enriched and standard-housed rats, but it appears to have little or no effect in rats reared in isolation (Yildirim et al., 2012). These differences could reflect significant changes in serotonergic function between EC and IC rats brought about by the rearing process, which was investigated in the current study.

### **Effects of Differential Rearing on Depressive-Like States**

The effects of environmental enrichment on FST performance are inconsistent and remain unclear, from having no effect (Cui et al., 2006), to the production of antidepressant-like states (Brenes et al., 2008; 2009). Cui et al. (2006) did not find an antidepressant-like effect of enrichment after 30 days of rearing during postnatal days 22-52. Brenes et al. (2008) found that enriched rats displayed decreased immobility and increased escape-directed behavior compared to IC or SC rats, but the rats were housed in their respective environmental conditions for 84 days before the FST. Brenes (2009) implemented a shorter rearing period and found that SC and IC rats displayed increased immobility from the pretest to the test session, but EC rats did not



display a significant change in immobility between the two tests. Compared to isolates, it has been found that rats reared in an enriched environment for six weeks display increased escape-directed (antidepressant) behavior and decreased immobility (depressive behavior) in the FST (Konkle et al., 2010).

Regardless of the promising potential of enrichment to attenuate depressive-like states, it remains that antidepressant effects of enrichment may only be evident after a longer rearing period. Moreover, the effects of isolation rearing on depressive-like states are inconsistent, from reducing immobility after 4 weeks of isolation (Wongwitdecha et al., 2006), to increasing immobility after 4 weeks, with a greater increase in immobility found after 8 weeks (Heritch et al., 1990). In another study, 4 or 8 weeks of environmental enrichment did not appear to prevent despair or depressive-like behaviors displayed in Huntington's disease-transgenic mice (Renoir et al., 2013). Likewise, and more relevant to the current study, Yildirim et al. (2012) observed that 6 weeks of environmental enrichment did not produce antidepressant-like effects, such that escape-directed behaviors were not different between SC and EC rats, and time spent immobile was actually significantly longer in EC and IC rats compared to SC rats.

### **Pilot Study Results: 10 mg/kg Fluoxetine in EC and IC rats**

A pilot study investigated the effects of differential rearing and fluoxetine (10 mg/kg, i.p.) administration on locomotor behavior and FST performance. Male Sprague-Dawley rats arrived at the lab at 21 days of age and were randomly assigned to an enriched (EC) or isolated (IC) condition where they were differentially reared for 30 days. The effect of fluoxetine (10 mg/kg, i.p.) on locomotor behavior was assessed during a 15-minute locomotor test session. Fluoxetine attenuated locomotor behavior in both EC and IC rats. The effect of fluoxetine on FST performance was then assessed. Rats were subjected to a 15-minute pretest immediately

followed by 3 injections of fluoxetine (10 mg/kg, i.p.) or vehicle 23.5 hours, 5 hours, and 1 hour before the 5-minute test session. All rats displayed an increase in immobility from the pretest to the test session, indicating that we did observe typical results. Fluoxetine did not, however, decrease immobility in either EC or IC rats compared to vehicle, suggesting that fluoxetine (10 mg/kg, i.p.) administered sub-chronically does not produce antidepressant-like effects in enriched or isolated rats. Interestingly, EC-vehicle and EC-fluoxetine rats exhibited more immobility than their IC counterparts.

### **Experimental Plan and Hypotheses**

The present study determined if differential rearing alters fluoxetine-induced performance in the rat forced-swim test (FST). We conducted behavioral studies examining the effects of fluoxetine (20 mg/kg) and environmental enrichment on locomotor behavior and depressive-like states in rats reared in an enriched (EC), isolated (IC), and standard (SC) condition. The established antidepressant, fluoxetine, was administered and depressive-like states were observed and recorded through performance and expression of escape-directed behaviors and immobility in the forced swim test.

To determine the effects of a standard rearing period (30 days) and a well-established antidepressant drug, the current study investigated the effects of differential rearing and the commonly prescribed selective serotonin reuptake inhibitor (SSRI), fluoxetine, on locomotor behavior and FST performance in Sprague-Dawley rats.

The overarching research objective of the current study was to investigate the effects of the serotonergic alterations elicited by the rearing process, and how these alterations affect the function of fluoxetine in the expression of depressive-like states in rats.

#### ***Specific Aim 1***

Investigate the effects of fluoxetine (20 mg/kg) on locomotor behavior in differentially reared rats.

### ***Hypothesis***

We hypothesized that fluoxetine (20 mg/kg) would significantly reduce rat locomotor behavior in all three environmental conditions. Furthermore, we anticipated that EC-vehicle rats would display significantly less locomotor behavior than SC and IC-vehicle rats. Fluoxetine (20 mg/kg) would further facilitate this reduction of locomotor behavior, such that EC-fluoxetine rats would display significantly less locomotor behavior than SC- and IC-fluoxetine rats.

### ***Specific Aim 2***

Determine if different rearing environments can alter the expression of depressive-like states in the FST.

### ***Hypothesis***

We hypothesized that there would be differences between environmental groups in FST performance such that EC rats would display more escape-directed behavior and less immobility than SC and IC rats.

### ***Specific Aim 3***

Examine the effects of fluoxetine (20 mg/kg, i.p.) on depressive-like states in EC, SC, and IC rats.

### ***Hypothesis***

We expected EC-fluoxetine rats to display more escape-directed behavior and less immobility compared to SC- or IC-fluoxetine rats. In other words, we expected isolation rearing to blunt the antidepressant effects of fluoxetine and lead to no increases in swimming and no decreases in immobility. Furthermore, we expected that the enhanced serotonergic function in

EC rats would lead to more of an antidepressant effect of fluoxetine (decreases in immobility and increases in escape-directed behavior) compared to IC and SC rats.

## **CHAPTER 2 - General Method**

### **Animals and Housing Conditions**

Male Sprague-Dawley rats were ordered from a commercial vendor and arrived in the laboratory at 21 days of age. Rats were then randomly assigned to environmental conditions where they reared in the enriched (EC), isolated (IC) or standard (SC) condition for 30 days. EC rats were housed in a group of 10 in a large metal cage (60x120x45 cm) that was lined with bedding. Fourteen non-toxic objects (children's toys, PVC pipe, etc.) were placed in the cage. Seven of the objects were changed daily and all of the toys were changed weekly. EC rats were also handled daily during scheduled toy changes. IC rats were housed individually in hanging metal cages (17x24x20 cm) that had wire mesh fronts and bottoms, and solid sides. IC rats did not have novel objects and were not handled during the 30 day rearing period. SC rats were housed in pairs in standard plastic shoebox cages (20x43x20 cm) to provide a known laboratory standard for comparison (Guide, 2011). SC cages had bedding, wire tops, and these rats were handled weekly during scheduled cage changes. All rats were housed under a 12:12 hour light:dark schedule, and had ad libitum access to food and water throughout the experiment.

### **Locomotor Behavior Screen and Apparatus**

To ensure fluoxetine did not increase locomotor behavior, which could yield false-positive results in the FST, it was necessary to assess locomotor activity separately to determine if fluoxetine altered general activity. Interestingly, antidepressant drugs tend to decrease locomotor activity in an open field, while they increase escape-directed behavior in the forced swim test, effectively highlighting the different neural substrates underlying general locomotion

and forced swim test behavior (Slattery & Cryan, 2012). Twenty-four hours prior to the locomotor test session, a 30-minute habituation session occurred to diminish any effect of novelty on the test day. During the following session, rats were administered 20 mg/kg fluoxetine or vehicle (distilled water), i.p., 23.5 hours, 5 hours, and 1 hour before locomotor testing. Locomotor activity was measured by recording photobeam interruptions in a test chamber measuring 40.64 x 40.64 x 40.64 cm (Coulbourn Instruments, TruScan 2.01). The chambers had Plexiglas walls and a stainless steel floor covered with bedding. The total distance traveled (cm) during the session was recorded. The locomotor session was 15 minutes in duration, which matched the length of the pretest of the FST.

### **Forced Swim Test Apparatus**

Rats were placed individually into a glass cylinder measuring 20.32 cm diameter X 40.64 cm height containing water ( $25^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$ ). Cylinder water was deep enough to ensure that the rats' hind-paws could not touch the cylinder's bottom. The swimming sessions consisted of a 15-min pretest, followed 24 hours later by a 5 min test session. The dimensions, temperature, and further procedures are based on the original FST (Porsolt et al., 1977), the modified FST (Detke & Lucki, 1996; Lucki, 1997), and those described in a recent FST review (Slattery & Cryan, 2012). The dimensions of the modified forced swim test were implemented to better detect the efficacy of selective serotonin reuptake inhibitors (Cryan et al., 2002; 2005; Lucki, 1997). Since the current study administered fluoxetine, an SSRI, it was necessary to utilize the dimensions of the modified FST.

The FST sessions were recorded by a video recorder from the side of the cylinder and the animals' escape directed behaviors (swimming and climbing) and immobility were later scored manually by the experimenters. An experimenter was present in the room at all times during the

swim sessions. After successful completion of the FST, each rat was dried with a towel, placed in a warm enclosure, and then placed in a dry transport cage (20x43x20 cm) equipped with bedding, food, and water. Rats in the enriched (EC) and standard (SC) conditions were placed back in their home cages only after their cohort(s) completed the FST.

The 15 min pretest and 5 min test session occurred in a different room from the colony room. Therefore, rats were transferred to the anteroom of the FST room, where they waited for at least one hour to minimize the effect of arousal due to transportation. The rats had access to food and water during this waiting period before they began the forced swim test. After each session, the rats were removed from the water, dried with a towel, and placed in a warm enclosure. The cylinders were rinsed and refilled after each rat throughout the experiment, and they were cleaned, sanitized, and dried each day after testing (rats were run in squads over the course of 6 days).

## **Experimental Procedures**

The FST procedures were used to determine the effects of differential rearing and fluoxetine on the expression of depressive-like states. Rats underwent the 30-day rearing period described above. After rearing, rats were injected with fluoxetine (20 mg/kg) or vehicle and tested in the locomotor chambers prior to the FST.

At least three days after locomotor testing, rats underwent the 15 min pretest for the forced swim test. After the pretest, rats received the fluoxetine or vehicle injections three times: 23.5 hr, 5 hr, and 1 hr before the 5-min test session. This injection schedule was identical to the schedule used in the locomotor test and was implemented using a counterbalanced drug treatment between the locomotor test and the FST.

## **Drug Regimen: 23.5 Hr, 5 Hr, and 1 Hr before the FST**

The dose and drug regimen used in the current study was specifically chosen based on previous literature. The injection schedule has been well-established to provide a robust response to a wide range of antidepressant compounds (Slattery & Cryan, 2012). This regimen results in prolonged brain penetration of the drug under study, and mimics a state of sub-chronic drug exposure and continuously elevated drug concentration in the rat (Cryan et al., 2005). This regimen has yielded a significant antidepressant-like effect with fluoxetine at various doses (Cryan & Lucki, 2000a; 2000b; Page et al., 1999)

Therefore, the dose of 20 mg/kg fluoxetine was administered in the current study based on previous research that has yielded robust reductions in immobility and increases in escape-directed behavior (Cryan & Lucki, 2000a; 2000b; Detke et al., 1995; Jang et al., 2009). Thus, 20 mg/kg is recommended as an ideal dose to consistently observe an antidepressant-like effect in rats (Slattery & Cryan, 2012).

### **Group Assignment and Sample Size**

Each environmental group consisted of 20 EC, 20 IC, and 20 SC rats for a total of 60 rats (Table 1), with 30 receiving fluoxetine and 30 receiving vehicle control injections in a counterbalanced manner between the locomotor test and the FST.

## **CHAPTER 3 - STATISTICAL TESTS**

### **Locomotor Data**

A 2 X 3 mixed-factorial ANOVA was performed to evaluate Drug treatment (fluoxetine or vehicle) and Environmental condition (EC, SC, or IC) on total distance traveled (cm) during the locomotor test. Significant interactions were probed using multiple comparison tests to

compare the effect of drug within each environmental condition, and to compare environmental conditions within each drug treatment. All alpha levels were set at  $p < 0.05$ .

## **Forced Swim Test Data**

### ***Scoring***

The scoring method was based on the modified forced swim test, as described in detail by Slattery and Cryan (2012). The first five minutes of the pretest (1<sup>st</sup> FST session) was scored to determine the effects of differential rearing on initial FST performance. The 5-minute forced swim test session and the first 5 minutes of the pretest session were broken into 60, 5-sec bins in which the predominant behavior during each 5-sec period was scored as swimming, climbing, or immobility. This totaled 60 scores for each rat in each session. The dependent measure for all analyses was the expression of escape-directed behavior (swimming and climbing) and the behavioral despair measure of immobility. These behaviors were recorded and later scored by experimenters blind to drug treatment. These behaviors were also scored by two experimenters who established high inter-rater reliability in which their respective scores varied by no more than 10%.

### ***Data Analyses***

To ensure there was an increase in immobility from the pretest to the test session, the pretest was compared to the test session through multi-factorial analyses of variance (Brenes et al., 2009; Slattery & Cryan, 2012). During the pretest and the forced swim test sessions, to determine if significant differences existed in escape-directed behavior or immobility, one-way ANOVAs were used with rearing condition (EC, IC, and SC) and pharmacological group (fluoxetine or vehicle control) as the between-subjects factors. Dependent measures (swimming, climbing, and immobility) were scored and separate ANOVAs were conducted for each



dependent variable. Significant main effects for pretest analyses were probed with multiple comparison tests using Tukey-Kramer adjustments to control for family-wise error rate ( $\alpha_{\text{FER}} = 0.05$ ). All alpha levels were set at  $p < 0.05$ .

Significant effects for the forced swim test session analyses were probed using a Bonferroni adjustment to control for family-wise error rate ( $\alpha_{\text{FER}} = 0.05$ ). A Bonferroni adjustment, rather than Tukey-Kramer, was appropriate for the following analyses given that not all of the possible pairwise comparisons were of interest in the current behavioral measure. Thus, for each of the following analyses with any significant effects, only a subset of all possible pairwise comparisons was investigated.

## CHAPTER 4 – RESULTS

### Locomotor Test (Figure 1)

For total distance travelled, the analysis revealed a significant main effect of drug treatment,  $F(1,54) = 151.59, p < .001$ , such that rats given fluoxetine ( $M = 944.53, SD = 655.93$ ) traveled significantly less than rats given vehicle control injections ( $M = 2907.86, SD = 1027.74$ ). Results also revealed a significant main effect of environmental condition,  $F(2,54) = 23.57, p < .001$ .

Furthermore, results also revealed a significant interaction between drug treatment and environmental condition on total distance travelled,  $F(2,54) = 5.94, p = .005$ . Fluoxetine decreased the total distance traveled in all three environmental conditions,  $F_s(1,54) = 18.48 - 74.86, ps < .01$ . EC-vehicle rats travelled on average 1187.4 cm more than the EC-fluoxetine rats. The SC-vehicle rats travelled on average 2312.9 cm more than the SC-fluoxetine rats. IC-vehicle rats travelled on average 3191.8 cm more than the IC-fluoxetine rats. Within the fluoxetine drug treatment, IC rats traveled significantly more than EC rats. However, both EC

and IC rats did not significantly differ in distance traveled from the SC rats. Comparing the vehicle conditions, IC and SC rats traveled significantly more than EC rats. However, the IC- and the SC-vehicle rats' total distance traveled did not significantly differ from each other.

### **Pretest**

To determine the effect of environmental condition on rats' escape directed behavior (swimming and climbing) or immobility during the first 5 minutes of the 15-minute pretest, three separate one-way analysis of variance (ANOVA) tests were conducted to compare EC, IC, and SC rats on each of the three pretest behaviors: swimming, climbing, and immobility.

#### ***Pretest Swimming (Figure 2)***

To assess the effect of environmental condition on swimming during the pretest, a one-way factorial ANOVA was conducted. There was a significant main effect of environmental condition on swimming during the pretest,  $F(2,55) = 3.77, p = .029$ . Using Tukey-Kramer adjusted multiple comparison tests, IC rats exhibited significantly less swimming ( $M = 15.25, SD = 5.71$ ) than SC rats ( $M = 21.25, SD = 7.17$ ). EC rats ( $M = 15.89, SD = 9.50$ ) displayed similar swimming to the IC rats which was less swimming compared to SC rats, but the difference was not statistically significant.

#### ***Pretest Climbing (Figure 3)***

To assess the effect of environmental condition on the other escape-directed behavior of climbing during the pretest, a second one-way factorial ANOVA was conducted. There was a significant main effect of environmental condition on rats' pretest climbing,  $F(2,55) = 4.66, p = .014$ . Using Tukey-Kramer adjusted multiple comparison tests, EC rats ( $M = 21.44, SD = 7.36$ ) displayed significantly more climbing during the pretest than IC rats ( $M = 15.75, SD = 8.32$ ) and

SC rats ( $M = 15.00$ ,  $SD = 5.11$ ). SC rats and IC rats did not significantly differ in the amount of climbing behavior during the pretest.

### ***Pretest Immobility (Figure 4)***

To test the effect of environmental condition on the amount of immobility displayed during the pretest, a one-way factorial ANOVA was conducted. There was a significant main effect of environmental condition on rats' pretest immobility,  $F(2,55) = 3.57$ ,  $p = .035$ . Using Tukey-Kramer, however, none of the environmental conditions were significantly different from each other.

### **Test Session**

To test the effects of drug and environmental condition on rats' depressive or antidepressant behavior during the forced swim test, three separate 2 (Drug treatment) x 3 (Environmental condition) factorial ANOVA tests were conducted to determine the main effect of environmental condition (EC, IC, and SC) and drug treatment (fluoxetine and vehicle) as well as the interaction effect of environmental condition and drug treatment on each of the three forced swim test session behaviors: swimming, climbing, and immobility.

### ***Test Session Swimming (Figure 5)***

To determine the effect of environmental condition and drug on rats' swimming behavior during the forced swim test session, a 2 (Drug treatment) x 3 (Environmental condition) two-way factorial ANOVA was conducted. Results revealed a significant main effect of drug treatment on swimming,  $F(1,51)=16.41$ ,  $p<.001$ . There was no significant main effect of environmental condition on rats' swimming behavior.

Rats treated with vehicle control injections ( $M = 16.21$ ,  $SD = 7.94$ ) interestingly displayed more swimming than rats treated with fluoxetine ( $M = 8.31$ ,  $SD = 7.78$ ). There was

also a significant interaction between environmental condition and drug treatment on rats' swimming in the test session,  $F(2,51) = 3.46, p = .039$ . To investigate this significant interaction effect further, nine multiple comparison tests were conducted to compare the effect of drug within each environmental condition (3 comparisons) as well as the effect of environmental condition for each drug treatment (3 comparisons per drug treatment = 6 comparisons). Using a Bonferroni adjustment, the nine multiple comparison tests indicated only one significant difference in swimming in EC rats receiving fluoxetine and EC rats receiving vehicle control injections. Specifically, EC-vehicle rats swam significantly more ( $M = 18.00, SD = 7.56$ ) than EC-fluoxetine rats ( $M = 3.70, SD = 2.54$ ).

#### ***Test Session Climbing (Figure 6)***

A 2 (Drug Treatment) x 3 (Environmental Condition) factorial ANOVA on climbing in the forced swim test session yielded no significant main effects of drug treatment or environmental condition, as well as no significant interaction, such that there were no significant differences in climbing in rats receiving fluoxetine or rats receiving vehicle in any of the three environmental conditions.

#### ***Test Session Immobility (Figure 7)***

A 2 (Drug treatment) x 3 (Environmental condition) factorial ANOVA on immobility behavior in the forced swim test session yielded no significant main effect of drug treatment or environmental condition. Rats' immobility during the forced swim test session did not differ between drug treatments or environmental conditions. Furthermore, there was no significant interaction between drug treatment and environmental condition; there were no significant differences in immobility in rats receiving fluoxetine or rats receiving vehicle in any of the three environmental conditions.

## **Behavioral Changes from the Pretest to the Test Session**

To investigate the alterations in rats' escape-directed behavior (swimming and climbing) and immobility from the first five minutes of the pretest to the five minute test session, three mixed-factorial repeated measures ANOVAs were conducted with repeated measures on session to compare changes in EC, SC, and IC rats' swimming, climbing, and immobility from the pretest to the test session. These analyses were run to validate the forced swim test paradigm and confirm the ability of the pretest to facilitate immobility and alter escape-directed behavior in the test session.

### ***Session Effect on Swimming***

A repeated measures ANOVA with repeated measures on Session to compare changes in swimming behavior from the pretest to the test session revealed a significant main effect of Session,  $F(1,55) = 20.39, p < .001$ , such that rats swam significantly more in the pretest ( $M = 17.43, SD = 8.03$ ) than they swam in the test session ( $M = 12.36, SD = 8.74$ ). This suggests that rats were less likely to display the escape-directed behavior of swimming in the test session compared to the pretest.

### ***Session Effect on Climbing***

A repeated measures ANOVA with repeated measures on Session to compare changes in climbing behavior from the pretest to the test session revealed a significant main effect of Session,  $F(1,55) = 18.14, p < .001$ . Rats exhibited significantly more climbing in the first 5 minutes of the pretest ( $M = 17.21, SD = 7.61$ ) compared to their climbing behavior in the test session ( $M = 11.66, SD = 10.81$ ). Similar to the effect on swimming, rats' climbing behaviors decreased in the test session compared to the pretest, suggesting that the rats showed fewer escape-directed behaviors in the test session than in the pretest.

### *Session Effect on Immobility*

The repeated measures ANOVA revealed a significant main effect of Session,  $F(1,55) = 109.71, p < .001$ , such that rats displayed significantly more immobility in the test session ( $M = 36.46, SD = 10.82$ ) compared to their immobility in the pretest ( $M = 22.95, SD = 9.66$ ). Therefore, rats displayed more immobility during the test session than they did in the pretest, confirming the presence of more depressive-like behavior in the test session.

### **Exploratory Analyses**

Given that the vehicle control injections for the test session resulted in significantly more swimming than fluoxetine injections for the test session, we questioned whether this result was due to the possibility that rats that received the vehicle were apt to swim more in the pretest, too. To answer this question, an exploratory 2-way ANOVA with repeated measures on Session was conducted to determine if there was a significant interaction between test session drug treatment and the behavioral changes from the pretest to the test session. As was seen in previous analyses, the exploratory analysis revealed a significant main effect of Session,  $F(1,54) = 20.83, p < .001$ , such that rats exhibited more swimming in the pretest than in the test session. However, there was not a significant interaction between Session and Drug treatment, which suggests that though the vehicle rats did swim more in the test session than did fluoxetine rats, both groups of rats decreased their swimming from the pretest to the test session in similar amounts.

## **CHAPTER 5 - DISCUSSION**

The overarching aim of the current study was to determine if the efficacy of fluoxetine as an antidepressant could be altered by differential rearing. We hypothesized that there would be differences between environmental groups in FST performance, such that EC rats would display

more escape-directed behavior and less immobility than SC rats. The results of the pretest partially supported our hypothesis in that EC rats displayed more climbing behavior than both IC and SC rats. However, there were no significant differences in immobility found between any of the three environmental conditions in the test condition.

Furthermore, we hypothesized that EC-fluoxetine rats would display more escape-directed behavior and less immobility compared to SC- or IC-fluoxetine rats during the test. The results from the current study did not support this hypothesis. However, we did observe the ability of differential rearing to alter the efficacy of fluoxetine, such that environmental enrichment appeared to reverse the antidepressant effects of fluoxetine, and isolation appeared to blunt them. These results suggest that at the current dose, fluoxetine was not protective against depressive-like states in the FST. Interestingly, this may be due to the ability of differential rearing, specifically environmental enrichment, to alter the efficacy of fluoxetine.

### **Locomotor Test**

For the initial behavioral measure, the current study examined the effects of differential rearing and fluoxetine treatment on general locomotor activity (total distance traveled, cm). Comparing vehicle control groups, rats reared in an enriched environment traveled less than standard and isolated rats. These results are consistent with previous literature in that EC rats display less locomotor activity compared to rats reared in either isolated or standard conditions (Gill, et al., 2012, Arndt et al., 2014). Rats were habituated to the locomotor apparatus one day before testing, which reduced the likelihood of novelty as an explanation for the behavioral differences observed between enriched, isolated, or standard-housed rats.

The locomotor test also revealed that fluoxetine significantly decreased locomotor activity in all three environmental conditions. This well-established effect (Slattery & Cryan,

2012) highlights the different neural substrates underlying locomotion compared to forced swim test behavior, as antidepressants are known to increase activity during the FST. Importantly, the injection regimen of fluoxetine administered before the locomotor test (23.5 hours, 5 hours, and 1 hour beforehand) matched the regimen of fluoxetine administered to rats before the forced swim test. This significant decrease in locomotor activity after fluoxetine administration confirmed a robust drug effect in all three environmental conditions.

### **Pretest**

Because the current study involved differential rearing, an environmental manipulation prior to behavioral testing, it was deemed necessary to record and analyze escape-directed behavior and immobility during the pretest, which occurred before the sub-chronic administration of fluoxetine. In doing so, this allowed us to measure any alterations in initial forced swim test behavior as a result of environmental condition alone. Comparing these environmental conditions, we observed interesting differences in initial escape-directed behavior in the pretest. IC rats displayed significantly less swimming behavior than SC rats. This may suggest that IC rats, compared to SC rats, are more inclined to display fewer antidepressant-like states (swimming) when faced with a novel, immediate stressor such as inescapable forced swimming. The main effect of environmental condition on pretest immobility reflected a trend that IC rats displayed more immobility, the primary measure of behavioral despair, in the first five minutes of the pretest. However, multiple comparison tests revealed no significant differences between environmental conditions for the measure of immobility.

Furthermore, EC rats displayed significantly more climbing behavior during the first five minutes of the 15-minute pretest compared to both SC and IC rats, suggesting that EC rats were more inclined to display antidepressant-like states (climbing) when faced with an immediate



stressor. These findings are generally consistent with previous literature showing that enrichment can provide a protective effect against depressive-like states (Brenes et al., 2009), whereas isolation rearing has been shown to augment depressive-like states (Kokare et al., 2010).

### **Test Session (FST)**

There were significant behavioral changes observed from the pretest to the test session, such that rats exhibited more immobility and less escape-directed behavior in the test session compared to the pretest. This indicates that the forced swim test paradigm did in fact produce typical increases in behavioral despair from the pretest to the test session. Although there was a significant decrease in locomotor activity in fluoxetine rats, indicating that the dose of fluoxetine altered locomotor behavior, we did not observe a significant increase in swimming and concurrent decrease in immobility in fluoxetine rats in any of the environmental conditions in the FST. These results differ from what has been found in standard-housed (SC) rats given fluoxetine, in that fluoxetine at 20 mg/kg generally yields robust reductions in immobility and increases in escape-directed behavior (Cryan & Lucki, 2000a; 2000b; Detke et al., 1995; Jang et al., 2009).

Interestingly, the multiple comparisons test investigating the significant interaction between environmental condition and drug treatment on rats' swimming behavior in the test session revealed that EC-fluoxetine rats exhibited significantly less swimming than EC rats treated with vehicle control injections. This finding was contrary to our hypothesis and suggests that the efficacy of fluoxetine may be altered in rats reared in an enriched environment. It is unclear why enriched rats failed to exhibit the increase in swimming behavior typically seen as a result of SSRI treatment. However, Simpson et al. (2012) found that rats housed in an enriched environment and standard group-housed rats showed no decreases in immobility after

administration of the tricyclic antidepressant desipramine compared to isolated rats.

Desipramine inhibits the reuptake of norepinephrine and serotonin. Unfortunately, this prior study did not report swimming behavior and therefore it is difficult to determine if our observed effects of fluoxetine are consistent with the results observed with desipramine. The results do suggest that enriched rats, compared to those reared in isolation, may have altered serotonergic mechanisms that could lead to a dissimilar response to many antidepressant drugs that inhibit serotonin reuptake, such as fluoxetine.

The non-significant differences in climbing behavior observed in the current study between fluoxetine rats and vehicle rats are not atypical compared to previous literature. SSRIs, such as fluoxetine, are more commonly known to elicit their effects by decreasing immobility and increasing swimming, with no effect on climbing behavior (Cryan et al., 2005). Alterations to climbing behavior are typically observed after the administration of drugs that alter catecholamine transmission (Detke et al., 1995; Lucki, 1997). As stated in the current study, fluoxetine primarily alters serotonergic function, with less of an effect on the catecholamines dopamine, epinephrine, and norepinephrine. The non-significant interaction between environmental condition and drug treatment in the current study further implies that differential rearing does not change climbing behaviors between fluoxetine- and vehicle-treated rats.

### **Differential Rearing**

The effects of environmental enrichment alone on FST performance remain unclear, from no effect after 30 days of rearing (Cui et al. 2006) to the production of antidepressant-like states after 76 and 36 days of differential rearing conditions, respectively (Brenes et al. 2008; 2009). Likewise, the effects of isolation rearing on depressive-like states are inconsistent, from actually decreasing immobility after four weeks of rearing (Wongwitdecha et al. 2006) to increasing it

after 4 weeks, with even more immobility present after 8 weeks of isolation rearing (Heritch et al. 1990). The current study provides support that a differential rearing period of four to five weeks is enough to impact baseline FST behavior, but is not enough to influence the development of immobility typically observed during the second forced swim test session. Again, it should be noted that the differential rearing period of four weeks has become a standard rearing duration for rodents that yields profound, reproducible and robust behavioral changes between EC and IC rats (Green et al., 2010; Renner & Rosenzweig, 1987), including changes within the serotonergic system (Simpson & Kelly, 2011).

Neurochemical analyses conducted by Brenes and colleagues (2008) have shown that after 84 days of differential rearing, EC rats, compared to IC and SC rats, have enhanced 5-HT expression in the hippocampus and frontal cortex which correlates positively with swimming and negatively with immobility in the FST. This suggests that longer rearing periods may be accompanied by additional neurochemical changes that could impact subsequent behavioral measures such as the FST. One of these additional changes seen in EC rats may manifest in the form of enhanced neurogenesis compared to IC and SC rats. Interestingly, fluoxetine treatment and environmental enrichment have both been shown to augment cell-proliferation and cell survival within the hippocampus (Tanti et al., 2013). This may be one of the neurochemical mechanisms responsible for the alteration of fluoxetine's efficacy between environmental conditions following a longer rearing period.

### **Differential Rearing-Induced Changes in Serotonin**

Previous research has shown that intact and functioning 5-HT transmission is required for SSRIs to produce antidepressant effects in the FST (Page et al., 1999). According to previous literature, the dosing regimen of fluoxetine implemented in the current study alters 5-HT

transmission and results in prolonged brain penetration while mimicking a state of sub-chronic drug exposure with continuously elevated drug concentrations in the rat (Cryan et al., 2005). Furthermore, the half-life of fluoxetine varies if given acutely or chronically, with a half-life of 1-4 days after acute administration that increases to 4-6 days after chronic treatment (Altamura et al., 1994). It is unclear if differential rearing can alter the half-life and thus the efficacy of fluoxetine.

Furthermore, it is also unclear which postsynaptic serotonergic receptors are responsible for the mediation of the behaviors associated with the FST (Cryan et al., 2005). Several studies have examined the effects of 5-HT receptor subtype activation or deactivation and have found alterations in fluoxetine efficacy in standard-housed rats. Of the receptor subtypes tested, it appears that the 5-HT<sub>1A</sub> (Detke et al., 1995), 5-HT<sub>1B/1D</sub> (Lopez-Rubalcava & Lucki, 1998) and 5-HT<sub>2C</sub> (Cryan & Lucki, 2000b) receptors play the most significant roles in influencing the FST behaviors commonly measured after SSRI administration, such as swimming and immobility (Cryan et al., 2005). The current literature is lacking and further research is needed to clarify enrichment-induced alterations to these specific 5-HT receptor subtypes, and how those possible alterations could influence fluoxetine efficacy.

Limited research has explored the role of these receptor subtypes in locomotor activity. Research suggests that activation of the 5-HT<sub>2C</sub> receptor reduces locomotor activity (Hayes et al., 2009). Therefore, the current results suggest that fluoxetine may be attenuating locomotor activity via activation of the 5-HT<sub>2C</sub> receptor. Furthermore, given that fluoxetine attenuated locomotor behavior in all environmental conditions, it also suggests that the differential effect of escape-directed behavior and immobility during the FST may be due to rearing-induced differences at the 5-HT<sub>2C</sub> receptor.

To our knowledge, this is the first study directly investigating the effects of differential rearing, specifically environmental enrichment, on the efficacy of fluoxetine. Based on previous research involving the effects of differential rearing on serotonergic function, it was hypothesized in the current study that enriched rats would be equipped with intact and functioning 5-HT transmission. Furthermore, because of enrichment-induced neuronal alterations that mimic SERT inhibition (MacGillivray et al., 2012), and possible isolation-induced attenuation of 5-HT release (Bickerdike et al., 1993; Fone & Porkess, 2008) we expected EC rats to respond well to fluoxetine and thus exhibit more antidepressant-like states compared to their isolated counterparts receiving fluoxetine. However, we found the contrary. Our results suggest that enrichment alters serotonergic mechanisms in such a way that may reverse the antidepressant effects of fluoxetine, resulting in decreased swimming compared to vehicle rats in the enriched condition.

### **Limitations**

The current study provided support for the notion that differential rearing can impact the efficacy of fluoxetine. However, this study is not without its limitations. We noticed fairly significant weight loss in rats receiving fluoxetine. Particularly at the time of the forced swim pretest, the rats administered fluoxetine previously for the locomotor test weighed significantly less than rats given the vehicle at the time of locomotor test, indicating a long-lasting residual effect of fluoxetine administration encroaching upon the time of the FST measure. Brenes and colleagues (2008) found a positive correlation between heavier weight and immobility time comparing enriched and isolated rats, with heavy isolated rats displaying more immobility than lighter enriched rats. Upon further investigation of this effect, however, Brenes and colleagues (2008) concluded that regardless of the significant body weight differences among

environmental groups, their escape-directed behavior and immobility behaviors kept the same tendencies and group differences shown in their previous analyses of FST behavior. Similarly, in the current study, exploratory analyses found that although the rats' weights were significantly different just before the pretest in fluoxetine-locomotor rats compared to vehicle-locomotor rats, their pretest forced swimming behavior did not significantly differ.

Fluoxetine is the only medication approved by the Food and Drug Administration (FDA) to treat bulimia nervosa in humans, as it helps reduce binge-eating episodes (Romano, 2002). With rats on *ad libitum* access to food and water, fluoxetine administration during the locomotor phase may have significantly reduced eating episodes leading up to the forced swim test in the current study, which may have impacted subsequent FST performance, but more research is needed to investigate this possibility, as we did not directly measure individual food intake.

Another interesting finding, yet possible limitation of the current study, is that long-term handling (from postnatal day 28 until two months of ages) can cause depressant-like behaviors in the FST (Cannizzaro et al., 2002), illustrated by increased immobility time. Daily handling of enriched rats for about one minute each day from postnatal day 21 until postnatal day 51 and continuing throughout experimentation is one of the main components of our environmental enrichment paradigm. Furthermore, in the study conducted by Brenes et al. (2008), which showed an antidepressant-effect of rats reared in their enriched condition, there is no mention of daily handling throughout the rearing phase of their experiment. Therefore, it is unclear whether our implementation of daily handling served as a chronic mild stressor leading to more depressive-like behaviors in the FST, but this previous research should be taken into consideration when interpreting the effects of environmental enrichment, and concurrent handling, on FST behaviors between differentially reared rats.

Lastly, video software to detect the occurrence of escape-directed behavior is commercially available, but was not purchased and used in the current study. Although this technology may be helpful, it is not recommended when measuring the effects of SSRIs or other pharmacological interventions because video software has been known to show inaccuracies in distinguishing bouts of swimming from immobility (Bogdanova et al., 2013), which are two of the most important dependent measures in detecting SSRI efficacy. Therefore, we implemented the most commonly used and recommended scoring method in the literature (Slattery & Cryan, 2012) to allow us to compare our results to currently published studies.

### **Future Research**

For future studies, it would be interesting to see how differential rearing can alter the efficacy of not only SSRIs, but other commonly-prescribed antidepressants. Fluoxetine is merely one specific compound in one class of antidepressants. More robust increases in FST swimming behavior between EC, IC, or SC rats may be observed after administering drugs in different classes, such as selective norepinephrine reuptake inhibitors (SNRIs) or monoamine oxidase inhibitors (MAOIs).

Additionally, latency to the initial immobility period (Castagné et al., 2009) could also serve as a future dependent measure and it would be interesting to see if this latency to exhibit immobility could be altered by environmental conditions or various environment-drug interactions. As an exploratory analysis for the current study, neither environmental condition nor fluoxetine had an effect on the latency to show immobility. In other words, EC, IC, or SC-fluoxetine or vehicle rats did not significantly differ in the time it took them to display their first bout of immobility. It is important to note, however, that latency to immobility period, according to Castagné and colleagues (2009) provides a valuable behavioral marker for assessing

the effects of tricyclics and selective norepinephrine reuptake inhibitors (SNRIs), but is not useful when investigating the effects of the SSRIs fluoxetine and escitalopram, the former of which was used in the current study. Our analyses are consistent with these observations, but we may observe an interaction between differential rearing and drug treatment with the use of other antidepressants.

### **Implications and Contributions**

Despite these limitations, the current study adds to the existing literature investigating how certain environmental factors, such as differential rearing, can impact the efficacy of commonly-prescribed antidepressant medication. Results from the pretest revealed differences in escape-directed behavior (antidepressant states) after 30 days of differential rearing. Therefore, the current study not only provides evidence that differential rearing can alter baseline frequencies of escape-directed behavior, but can also lead to differences in the responsiveness to, and efficacy of, commonly prescribed antidepressant drugs such as fluoxetine.

The effects of environmental enrichment on FST behaviors are inconsistent, from having no effect (Cui et al. 2006) to producing antidepressant-like states (Brenes et al. 2008; 2009). Likewise, the effects of isolation rearing on depressive-like states are inconsistent, from actually reducing despair (Wongwitdecha et al. 2006) to increasing it (Heritch et al. 1990). The results of the pretest in the current study provide support that enrichment can lead to increases in the initial escape-directed behaviors of swimming and climbing. However, results from the test session in the current study suggest that fluoxetine efficacy may be blunted in isolated rats and reversed in environmentally enriched rats. Therefore, careful consideration of all environmental factors should be used when prescribing antidepressant medication.



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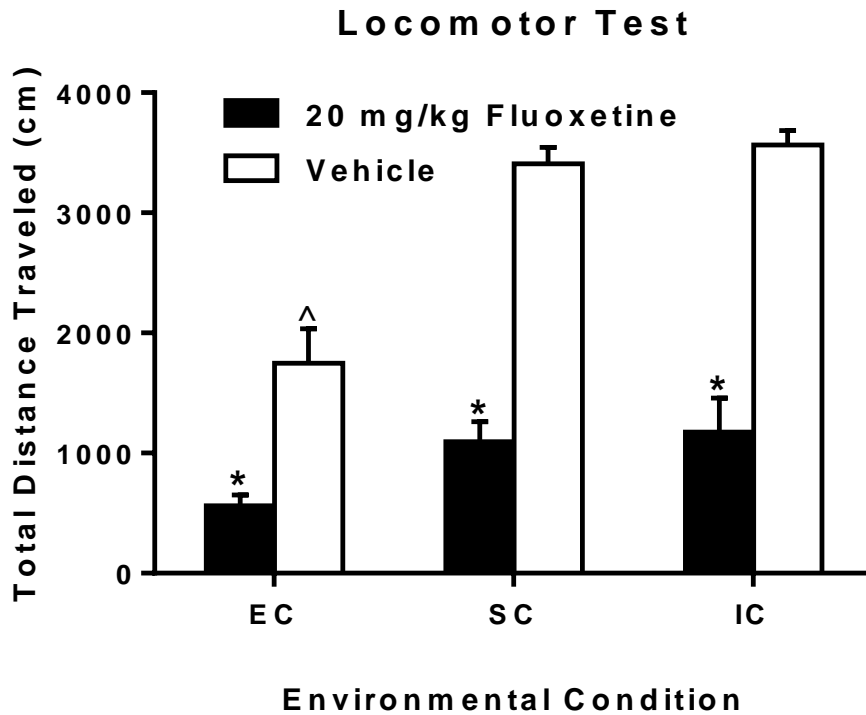
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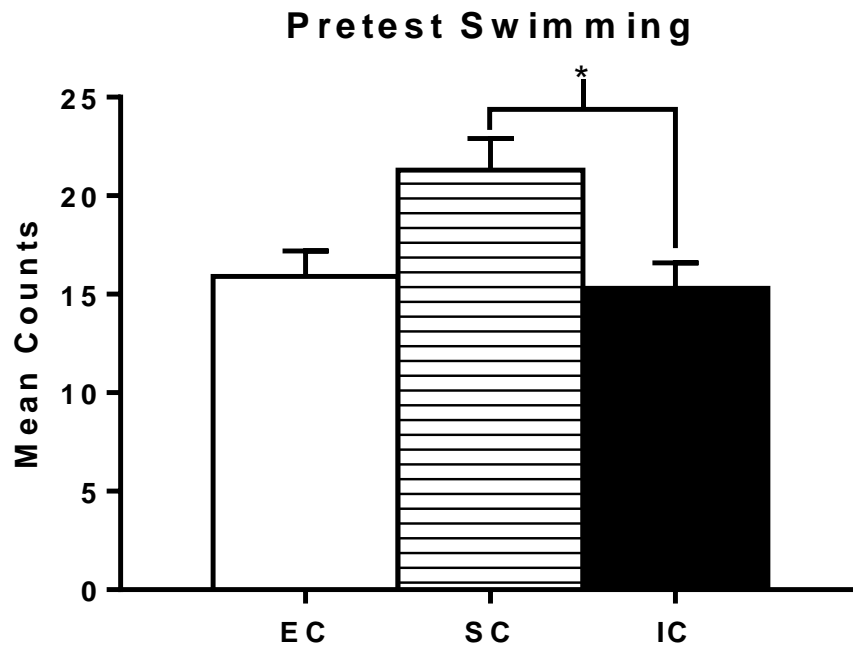


**Figure 1 Total locomotor distance (cm) traveled ( $\pm$ SEM) in enriched (EC), standard (SC), and isolated (IC) rats after fluoxetine (20 mg/kg, i.p.) or vehicle administrations 23.5 hours, 5 hours, and 1 hour before the locomotor test.**



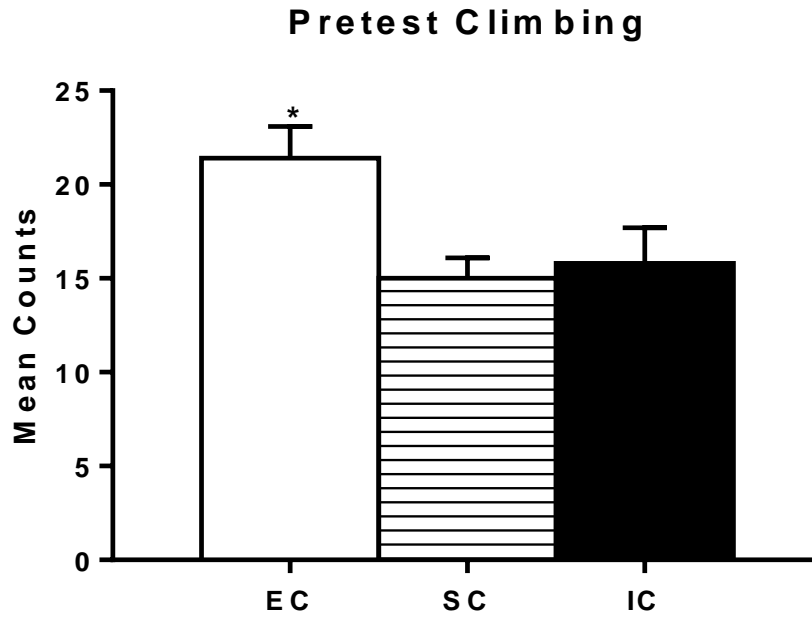
*Note.* Asterisks (\*) indicate significant reductions in locomotor activity in fluoxetine-treated rats compared to vehicle controls for all three environmental conditions. Caret sign (^) indicates significant reductions in locomotor activity in EC-vehicle rats compared to both SC- and IC-vehicle rats ( $p < .05$ ).

**Figure 2 Total mean counts ( $\pm$ SEM) of swimming behavior in EC, SC, and IC rats during the first 5 minutes of the 15 minute pretest.**



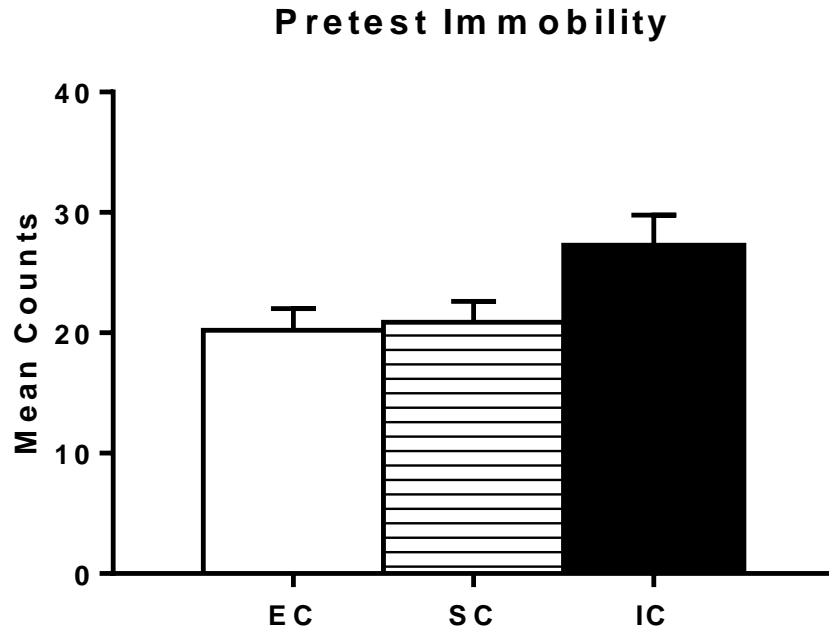
*Note.* The asterisk (\*) indicates that SC rats exhibited significantly more swimming than IC rats ( $p < .05$ ).

**Figure 3 Total mean counts ( $\pm$ SEM) of climbing behavior in EC, SC, and IC rats during the first 5 minutes of the 15 minute pretest.**

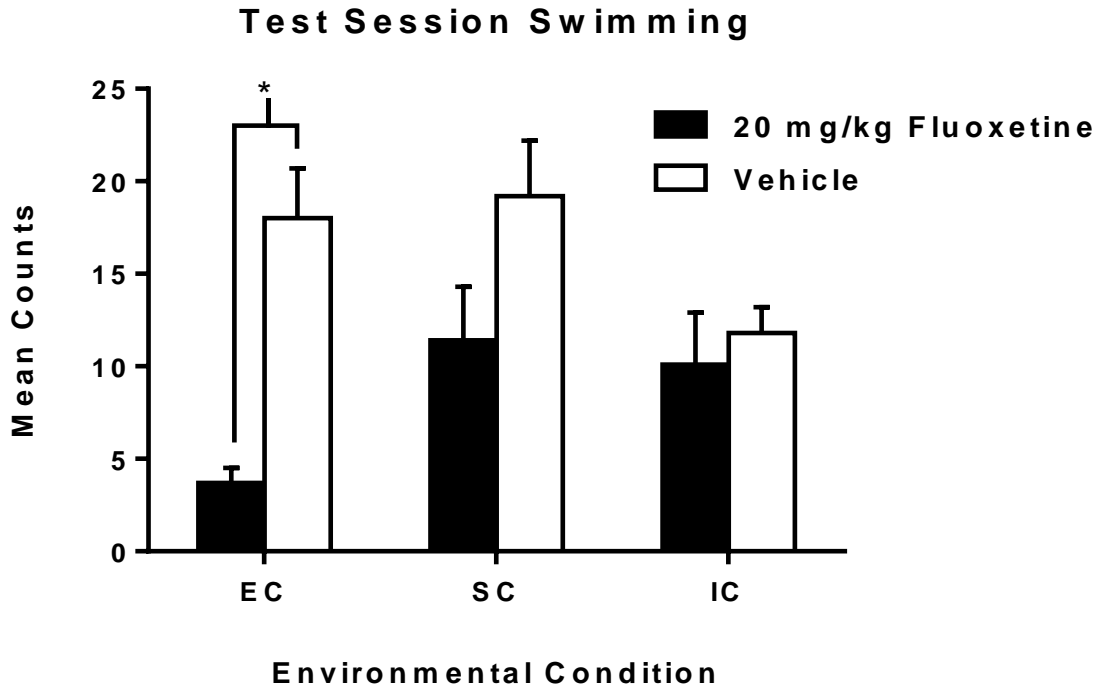


*Note.* The asterisk (\*) indicates that EC rats exhibited significantly more climbing than both SC and IC rats ( $p < .05$ ).

**Figure 4 Total mean counts ( $\pm$ SEM) of immobility behavior in EC, SC, and IC rats during the first 5 minutes of the 15 minute pretest.**

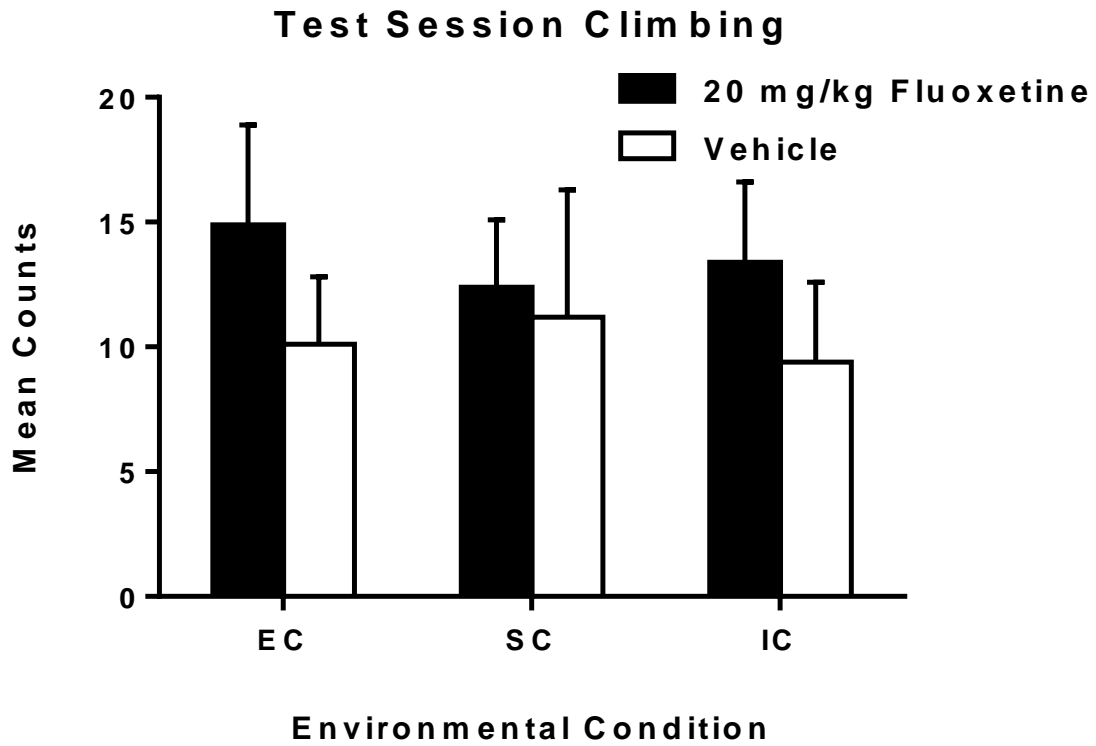


**Figure 5 Total mean counts ( $\pm$ SEM) of swimming behavior in rats given fluoxetine (20 mg/kg, i.p.) or vehicle 23.5 hours, 5 hours, and 1 hour before the forced swim test session.**

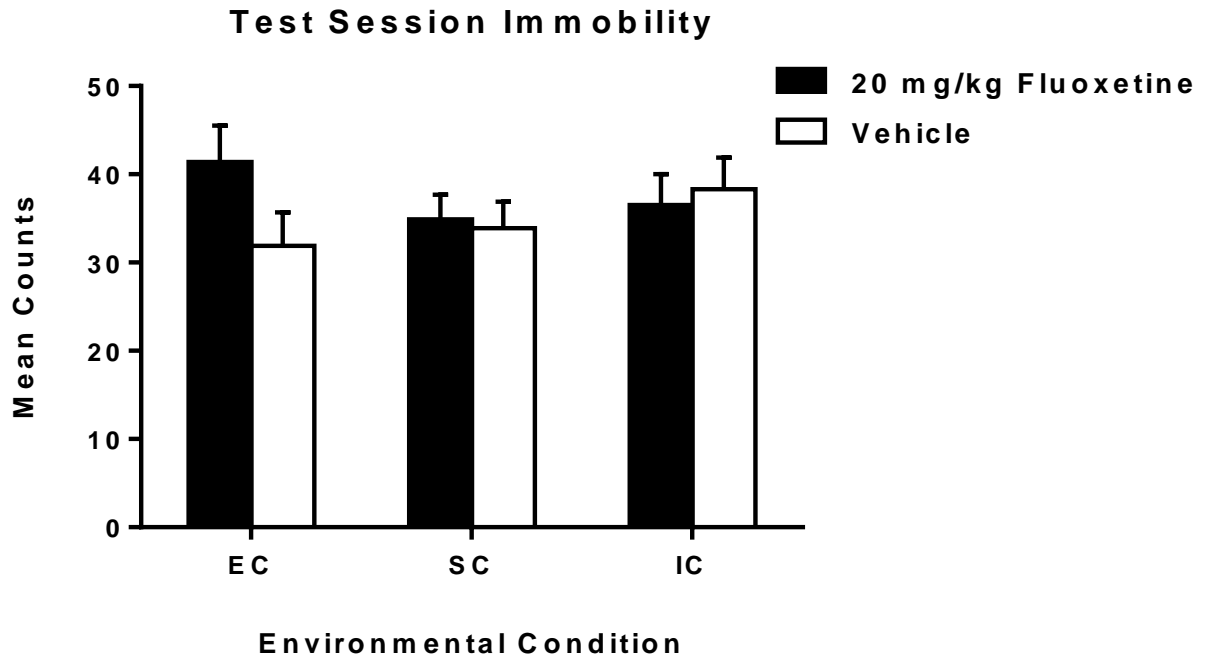


*Note.* The asterisk (\*) indicates that EC-fluoxetine rats exhibited significantly less swimming than EC-vehicle rats ( $p < .05$ ).

**Figure 6 Total mean counts ( $\pm$ SEM) of climbing behavior in rats given fluoxetine (20 mg/kg, i.p.) or vehicle 23.5 hours, 5 hours, and 1 hour before the forced swim test session.**



**Figure 7 Total mean counts ( $\pm$ SEM) of immobility behavior in rats given fluoxetine (20 mg/kg, i.p.) or vehicle 23.5 hours, 5 hours, and 1 hour before the forced swim test session.**



**Table 1 Fluoxetine (20 mg/kg, i.p.) and vehicle treatment groups and sample sizes for enriched (EC), isolated (IC), and standard (SC) rats**

<b>Drug Treatment</b>	<b>Environmental Condition</b>		
	EC	IC	SC
20 mg/kg fluoxetine	n=10	n=10	n=10
Vehicle	n=10	n=10	n=10