Equal But Opposite: Acute Anxiolytic versus Chronic Anxiogenic Effects Following Adolescent Fluoxetine Use

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By

Amber Rowse

University of Canterbury

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Abbreviations

ANOVA= analysis of variance

C = control group

E = early treatment group

EL = early plus late treatment group

EPM = elevated plus maze

FDA = United States Food and Drug Administration

Flx = fluoxetine (Prozac)

IP = intraperitoneal injections

L = late treatment group

5-HT = serotonin; 5-hydroxytryptamine

MDD = major depressive disorder

OCD = obsessive compulsive disorder

PO = oral administration

PND = postnatal day

S.E.M = standard error of measurement

SIT = social interaction test

SSRIs = selective serotonin reuptake inhibitors

TAD = treatment for adolescents with depression

UEEPM = unstable elevated exposed plus-maze

WKY = Wistar Kyoto rat

Abstract

Fluoxetine (Flx) is currently one the most commonly prescribed antidepressant medications for adolescents suffering from various emotional disorders. Flx has been approved by the United States Food and Drug Administration (FDA) for the treatment of adolescent major depressive disorder (MDD) and obsessive compulsive disorder (OCD), despite limited research into the possible long-term effects of this psychoactive substance on the developing adolescent brain. The current study aimed to investigate the effects of adolescent Flx exposure on later anxiety-like behaviour in rats. The current study involved the oral administration of Flx hydrochloride, via drinking water, to male and female adolescent PVG/C Hooded rats. There were three treatment conditions with differing Flx treatment periods (early, late or early plus late), and a control group. Immediately following Flx treatment, all groups were tested on an acute open-field test. The rats were then tested on two later occasions during adulthood on a battery of anxiety-related tests: the light-dark box test, the responsiveness to brightness change Y-maze and the social interaction test (SIT). On the acute open-field test, the Flx-treated rats displayed greater ambulation and possibly lower corner square occupancy, suggestive of an acute anxiolytic effect of Flx. The chronic effects testing suggested that rats treated during the late phase of adolescence, either alone or in combination with early treatment, were the most affected by Flx. The late Flx-treated group spent less time in the light during the light-dark box test, and defecated more than other groups during the SIT. In addition, the males within the late Flx-treated group spent significantly more time within the corner squares and less time within the centre squares of the SIT apparatus than males from the early Flx-treated group. Overall this study suggests that the long-term effects of adolescent Flx use are anxiogenic.

1.0 Introduction

1.1 General Overview

Fluoxetine (Flx) is currently one of the most commonly prescribed antidepressant medications for children and adolescents presenting with emotional disorders, and it's use appears to be increasing internationally (Zito et al., 2002; Aras, Tas & Unlu, 2006; Murray, de Vries & Wond, 2004). Despite approval by the United States Food and Drug Administration (FDA) in 2003 for the treatment of both child and adolescent major depressive disorder (MDD) and obsessive compulsive disorder (OCD), there has been ongoing speculation regarding the safety and efficacy of Flx for this younger population (Cohen, 2007; Garland, 2004). The majority of empirical research to date has focused on the acute effects following either a single dose or more chronic administration of Flx, and has predominantly focused on adults or prenatal effects. There has been a serious lack of research investigating the longer term sequelae associated with adolescent Flx use (Andersen & Navalta, 2004; LaRoche & Morgan, 2007). Research into the long term effects associated with adolescent Flx use is an important area of enquiry, given the widespread use of this drug and its potential to alter the developing adolescent brain.

1.2 Historical Context

Thalidomide was prescribed for the treatment of morning sickness in the 1950s. The discontinuation in the marketing of thalidomide following reports of limb abnormalities and other congenital defects in individuals exposed prenatally to this drug (Franks, Macpherson & Figg, 2004), sparked interest in the potential teratogenic effects of other psychoactive substances when taken during pregnancy. In accordance, most of what we currently know about the behavioural and neural correlates of drug use on the developing brain has come from research conducted on the prenatal period and to a lesser extent, the early postnatal period (Andersen &

Navalta, 2004). There has been very little research into the effects of drug exposure on the developing adolescent brain, with generalizations from adult research being made to adolescents. However, with increasing recognition that the adolescent brain is a developing structure, there has been an increased interest over the past decade in the effects of psychoactive drugs when taken during adolescence (Spear, 2000). Indeed a predominant focus within the adolescent-drug exposure literature has been on drugs of abuse, such as nicotine, ethanol and cocaine (Andersen, 2005; Smith, 2003). With the recognition at both a professional and societal level, that several psychiatric disorders occur in adolescents and the need for treatments that are both empirically supported and safe, more research into the enduring effects of prescription medications is required (Andersen & Navalta, 2004). When researching such enduring effects within a human clinical population it is particularly difficult to tease apart what effects are due to the medication and which are part of the disease process itself (Andersen & Navalta, 2004). This is where animal research can be helpful, with the potential for mapping the enduring effects of the drug on behavioural and neural correlates, without the confounding effects of the disease process itself.

1.3 Adolescence

Adolescence within humans can be defined as a transitory period in which an individual moves from a state of immaturity and dependence, to one of maturity and independence (Spear, 2007). Adolescence is not synonymous with puberty, but includes the physiological changes associated with puberty, as well as behavioural changes (Spear, 2000). Physiological changes include a growth spurt and increased levels of gonadal hormones such as estrogen and testosterone. Behavioural changes include increased novelty seeking, impulsivity, risk taking and peer affiliation (Spear, 2007a).

1.3.1 Adolescent Animal Models

Although initially conceptualized as a developmental phase unique to humans, the behavioural and physiological changes noted above as characteristic of adolescence have been documented in a range of other mammalian species including rodents and nonhuman primates (Spear, 2000). The acceptance of adolescence as a developmental phase in rats has allowed for the development of animal models of adolescence. Although the human brain is more complex than the rat brain, parallels in brain anatomy and processes are observed (Spear, 2007). These animal models have furthered our understanding of the developing adolescent brain, and also allowed for the investigation of potentially teratogenic drugs during this period (for example; Aitchison & Hughes, 2006; Anderson & Hughes, 2008; Smith, 2003). As noted above, the use of animal models also allows us to map the effects of drug exposure alone, without the confounding effects of the disease process.

1.3.2 Timing of Adolescence

The boundaries of what we can define as "adolescence" are imprecise across species (Spear, 2007). Within humans, adolescence is generally regarded as the period from approximately ages 10 to 20 years (Spear 2007a). In rats, the adolescent period has been broadly defined as postnatal day (PND) 28 through to PND 42. Spear (2007) suggests monitoring rats from early post-weaning (approximately PND 25) through until PND 55, in order to be sure of encompassing the entire adolescent period in both sexes. It is noteworthy that there is great variability in the timing of various aspects of adolescence between individuals, as well as sex differences (Spear, 2007a). Across species, females tend to show characteristics of adolescence earlier than males, evidenced by earlier pubertal changes (Spear, 2007a).

1.3.3 Adolescent Brain Development

Despite extensive research into the behavioural and physiological changes during adolescence, only more recently has the research focused on the neuroanatomical changes associated with adolescence (Spear, 2007). The advent of brain-imaging and its application to 'normally' developing adolescents has extended our knowledge within this area substantially (Paus, 2005), and there is now a growing consensus that the adolescent brain is still developing (Giedd, 2004).

The brain size does not change dramatically during adolescence, with the human brain reaching 90% of its total adult size by age six years (Casey, Tottenham, Liston & Durston, 2005). However, the organization of the brain is adjusted throughout adolescence (Giedd, 2004). Through the process of myelination, white matter increases linearly throughout childhood and adolescence, with similar rates documented across the four brain lobes (Giedd et al., 1999). White matter consists of myelin, a fatty substance produced by oligodendrocytes which serves to increase the speed of neural signals, thus information processing, within the brain (Giedd et al., 2009).

During adolescence the brain is also 'remodelled' through the process of synaptic pruning. This process involves the overproduction and subsequent selective pruning of synapses in both a region- and sex- specific manner (Andersen & Navalta, 2004; Spear, 2007). It is thought that axonal connections which an individual has used will be strengthened, while others will be eliminated if not used (Casey et al., 2005).

Although the processes detailed above occur widely across the brain, there are particular brain regions which undergo more dramatic changes than others. The prefrontal cortex and limbic

system are the brain regions in which the most substantial changes take place, whereby shifts in the balance between the mesocortical and mesolimbic dopaminergic systems takes place (Spear, 2000). Spear (2007) notes that up to 50% of neural connections are lost within cortical regions of the brain, with the most substantial synaptic pruning occurring within the prefrontal cortex.

There is variation in the timing in which synapses are produced and then eliminated within particular areas of the cortex. Andersen (2003) notes that the synaptic density peaks within the primary visual cortex much earlier than within the prefrontal cortex. In fact, the evolutionarily later developing structures are those which are the latest to be remodelled. Consequently, given the cortex and in particular the prefrontal cortex are the most evolutionarily advanced, these are the areas which are remodelled last. Andersen (2003) notes that these neuroanatomical changes map onto the functional developments within each system. For example, motor development occurs before cognitive development, which parallels the development of the striatum and cortex, respectively. In addition, synaptic density changes are thought to occur in parallel with receptor density changes.

1.3.4 Serotonergic System Development

de Jong et al. (2006) documented the changes within the serotonergic system during rat adolescence. These changes include increased 5-HT release within the raphe nuclei, and increased binding to the 5-HT$_{1A}$ receptor within the hippocampus, cerebral cortex, midbrain and brainstem. In addition, 5-HT levels increase within the hippocampus, striatum, brain stem and cortex (de Jong et al., 2006).

Moll et al. (2000) mapped the age-related changes in the densities of presynaptic monoamine transporters across different regions within the rat brain. These investigators found that within the rat frontal cortex, the density of 5-HT transporters progressively increased throughout the investigated age range (from PND 25 through to PND 240). Within the striatum, midbrain and brain stem the densities of 5-HT transporters remained at the same level from PND 25 onwards. The authors explained these findings as the age-associated changes in receptor densities being more prominent in the more phylogentically younger brain regions.

1.3.5 Neuronal Imprinting

Andersen (2003) discussed a host of both intrinsic and extrinsic factors which regulate brain development. Intrinsic factors included the trophic role of neurotransmitters, transient receptor expression, pharmacological sensitivity to drugs and sex differences. Extrinsic factors included experience expectant and experience dependent factors, as well as neuronal imprinting. Experience expectant factors refer to the critical periods during development, in which particular environmental stimulation is required for normal development. Experience dependent development refers to the sensitive periods during development. During a sensitive period, particular stimuli may alter neuronal development in ways which may affect later functioning.

Neuronal imprinting refers to the enduring effects on the central nervous system following exposure to psychoactive substances. As stated by Andersen and Navalta (2004) "drug effects incubate" (p423), whereby immediately following drug exposure, behavioural and/or neural effects may not be found. However, delayed effects may be found later on in life. Andersen and Navalta (2004) made an association between these delayed effects and the maturational changes within the brain. More specifically, brain systems most affected by drug exposure may be those

which are undergoing changes at the time of exposure. However, the effects on this vulnerable brain system may not be expressed until this system reaches full maturation. Andersen (2003) suggests that alterations in the level of a neurotransmitter will lead to subsequent region-specific alterations. In addition, Andersen (2003) notes that the transient expression of various receptors may play a trophic role.

1.3.6 Adolescent Drug Exposure: What We Know About Other Drugs

Our current knowledge of the effects of drugs on the developing adolescent brain and later behaviour, has come from a predominant focus on drugs of abuse such as nicotine, alcohol, and opiates (Smith, 2003). In addition, recent studies have documented the long-term effects associated with substances such as 1-benzylpiperazine (BZP; Aitchison & Hughes, 2006) and caffeine (Anderson & Hughes, 2008). Aitchison and Hughes (2006), for example, found that PVG/C Hooded rats treated with BZP during late adolescence (PND 45-55) displayed heightened anxiety during behavioural testing in early adulthood. These results were interpreted as a possible reflection of altered forebrain serotonergic pathways, possibly in the form of increased 5-HT transporter densities.

1.3.7 Psychiatric Disorders During Adolescence

Perhaps related to the characteristic physiological and behavioural changes, adolescence is a period in which the onset of several psychiatric disorders occurs, with prevalence rates rising during this developmental phase (Barlow & Durand, 2005). The prevalence of Major Depressive Disorder (MDD) increases dramatically during adolescence, with rates ranging from 4% to 8% of those within the general population (Emslie et al., 2002). For individuals diagnosed with MDD during adolescence, pharmacological treatment is often employed either alone or in

conjunction with other therapeutic strategies. The most commonly prescribed antidepressant medications are currently tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs; Murray et al., 2004). In one United Kingdom sample of individuals aged 18 years or less, it was found that 19.5% of antidepressant prescriptions between 1992 and 2001 were for Flx (Murray et al., 2004).

1.4 Anxiety

Anxiety in humans can be defined by a unique a set of physiological, cognitive and behavioural symptoms (Westbrook, Kennerley & Kirk, 2007). The physiological correlates of anxiety reflect activation of the autonomic nervous system and may include symptoms such as muscle tension, increased heart rate and increased perspiration. The cognitive symptoms of anxiety typically involve information processing which is bias toward threat. The behavioural symptoms of anxiety include withdrawal and avoidance (Westbrook et al., 2007).

The concept of 'anxiety' in rodents is based on the assumption that anxiety in humans and animals is comparable (Ohl, 2003). When placed in unfamiliar environments, rats display a series of anxiety-like responses. Initially, the rat may show decreased exploratory behaviour, while freezing, defecating and urinating to a greater extent. Rats may also engage in risk assessment behaviour (Palanza, 2001). Risk assessment behaviours include sniffing, stretched back postures and scanning for danger (Rogers, Cao, Dalvi & Holmes, 1997).

1.4.1 Animal Models of Anxiety

There are several models of anxiety in animals, and these models can be classified into conditioned or unconditioned models (Rogers et al., 1997). Conditioned models typically involve

some form of food or water deprivation, or electric shock, and involve a great deal of training for the animals. Examples of conditioned models include the four plate test or defensive burying tests. Unconditioned models, on the other hand, draw upon natural unlearned tendencies of the animal and do not require any deprivation, shocks or training. Naturally occurring situations which invoke an anxious response in both mice and rats include unfamiliar open spaces, bright light and heights (Palanza, 2001). A variety of unconditioned animal models of anxiety have been developed which draw upon these naturally aversive situations. A few of the more common tests are detailed below:

- *Open-Field Test:* the open-field test draws upon the rat's natural conflict between the tendency to explore novel environments, and their aversion to wide open spaces. As originally constructed the open-field test was a large circular apparatus, but more common now is a large square apparatus with sides high enough so that escape is not possible. The open-field test provides measures of both locomotor activity and anxiety, based on various ethological recordings as well as spatiotemporal measures, specifically, the relative movement of the rat between the inner and outer squares of the apparatus (Prut & Belzung, 2003).
- *Elevated Plus Maze (EPM):* the EPM is one of the most widely used animal models of anxiety, which draws upon rats' innate fear of both open and elevated spaces. The apparatus for the EPM is a plus shaped maze positioned above the ground, with two enclosed and two open arms (Borsini, Podhorna & Marazziti, 2002). A more anxious rat tends to avoid the open arms and spend most of its time within the enclosed arms (Silva & Brandao, 2000).
- *Social Interaction Test (SIT):* the SIT draws upon rats' aversion to bright light and unfamiliar situations, and involves the placement of two unfamiliar same-sex rats within

an open-field arena. Anxiety is measured in terms of the amount of time the rats spend interacting, with a reduction in interaction time thought to reflect heightened anxiety (File & Seth, 2003).

- *Light-Dark-Box Test:* the light-dark-box test draws upon the natural tendency of a rat to explore novel environments, but also their aversion to bright lights. The apparatus for the light-dark box test, as the name suggests, is a shuttle box with one side that is dark, and the other side brightly lit. Heightened anxiety is evidenced by reduced entries into and time spent within the light side of the box (Bourin & Hascoet, 2003).

1.4.2 Sex Differences in Anxiety

Sex-related discrepancies have been reported for some anxiety disorders, notably, females are over-represented in those diagnosed with generalized anxiety disorder (GAD), social anxiety disorder (SAD) and specific phobias (Palanza, 2001). Despite this higher prevalence in females, the animal literature has predominantly utilized male subjects. Amongst the few rat studies which have used both sexes, there has been a trend toward males defecating more and exhibiting less locomotor activity relative to females. These results have tended to be interpreted as evidence of heightened emotionality in males (Palanza, 2001).

As outlined by Palanza (2001), many of the animal models of anxiety have been developed using male subjects only, and it is therefore not clear how well they model anxious behaviour in females. Johnston and File (1991) accordingly studied the behavioural profiles of male and female Lister rats in three tests of anxiety: the SIT, EPM and the Vogel conflict test. These authors concluded that no firm conclusions could be drawn regarding which sex was more anxious, as the results of these three behavioural tests were not in the same direction. For

example, the results of the SIT suggested the females were more anxious than males, as they displayed relatively lower levels of interaction. Conversely, in the EPM, the females displayed less aversion to the open arms, indicative of reduced anxiety.

1.4.3 Serotonin Hypothesis of Anxiety

5-HT has long been recognised as playing an important role in anxiety (File & Seth, 2003). As outlined by Drapier et al. (2007), the serotonin hypothesis of anxiety postulates that increased 5-HT levels are associated with anxiogenic (anxiety-like) effects, while reductions in 5-HT are associated with anxiolytic (anxiety-reducing) effects. The initial clinical effect of Flx is often reported to be an exacerbation of anxiety, presumably due to the initial increase in 5-HT associated with Flx. However, the longer-term effects, although not well understood, reportedly lead to inhibited 5-HT neuronal firing (Silva, Alves & Santarem, 1999).

5-HT is one of the monoamines within the central nervous system and plays a role in mood, eating, sleeping, arousal and the regulation of pain (Carlson, 2007). Serotonergic cell bodies are positioned in nine clusters within the brain, predominantly situated in the raphe nuclei of the midbrain, pons and medulla (Carlson, 2007). The neurons from within the raphe nuclei have widespread connections to various regions of the brain associated with emotion such as the hypothalamus, hippocampus and cortex (see section 1.4.4 below). More specifically, there is a cluster of serotonergic cell bodies in both the dorsal and medial raphe nuclei. The axons of the neurons in the dorsal raphe nuclei project to the cerebral cortex and basal ganglia. Those in the medial raphe nuclei project also toward the cerebral cortex, but additionally toward the dentate gyrus (Carlson. 2007).

1.4.4 Neuroanatomical Correlates of Anxiety

The literature documenting the neuroanatomical correlates of anxiety is extensive. With advances in neuroimaging and neurochemical analysis, research is turning toward profiling the neuroanatomical and neurochemical correlates of specific anxiety disorders. However, several neuroanatomical regions have been implicated in fear and anxiety behaviours in general. Most notably, the set of brain structures labelled as the limbic system, which is primarily involved in the processing of emotion and motivation, has been implicated in anxiety. The limbic system is comprised of several interconnected areas, namely, the hippocampus, amygdala, fornix, mammillary bodies and the limbic cortex (Carlson, 2007). The hypothalamus, recognised for its role in the modulation of the autonomic nervous system, has also been implicated in anxiety (Charney, 2003). In addition, several cortical areas have been implicated in anxiety, including the insular cortex as well as the orbital and medial prefrontal cortex, which have reciprocal projections with the amygdala. These reciprocal projections allow the prefrontal cortex to modulate amygdala-mediated responses, and also allow the amygdala to modulate prefrontal responses (see Charney, 2003 for review).

1.5 Fluoxetine

Flx is approved by the United States Food and Drug Administration (FDA) for the treatment of a number of psychiatric conditions in adults, including MDD, Obsessive Compulsive Disorder (OCD), Bulimia Nervosa and Panic Disorder (Leslie, Newman, Chesney & Perrin, 2005). In addition to approved use in adults, Flx has recently been approved for the treatment of childhood and adolescent (ages 7-18 years) MDD and OCD (Safer, 2006).

1.5.1 Pharmacological Action

Flx (Prozac) is one of the class of drugs termed 'selective serotonin reuptake inhibitors' (SSRIs). In addition to Flx, other SSRIs currently used include paroxetine (Paxil), sertraline (Zoloft), fluvoxamine (Luvox) and citalopram (Celexa; Julien, 2001). SSRIs are serotonin (5-HT) agonists, acting to increase the amount of 5-HT within the synaptic cleft, thus increasing the amount of serotonin available to activate post-synaptic receptors. As the name suggests, SSRIs block the actions of the presynaptic transporter, inhibiting the reuptake of 5-HT into the presynaptic cell (Julien, 2001). SSRIs reportedly do not appear to block the reuptake of other neurotransmitters (Beasley, Masca & Potvin, 1992).

Secondary effects at the receptor level occur following more chronic administration of Flx. More specifically, the increased extracellular 5-HT leads to both autoreceptor and postsynaptic receptor desensitisation (Andersen & Navalta, 2004). 5-HT release from nerve terminals is regulated by somatodendritic 5-HT1A autoreceptors. When these autoreceptors are activated by 5-HT itself, they provide a negative feedback loop to reduce the release of further 5-HT. Following chronic Flx administration, however, these autoreceptors become desensitised due to overstimulation, which leads to an increased 5-HT level within the synapse (Silva & Brandao, 2000).

1.5.2 Clinical Efficacy

Clinically, Flx has been shown to have anxiolytic effects following chronic administration. However, acute administration of Flx tends to produce an anxiogenic profile (File & Seth, 2003). Earlier investigations into the efficacy of Flx for the treatment of pediatric and adolescent depression showed limited success. These earlier studies showed similar rates of improvement

across the treatment and placebo groups, suggesting a significant placebo-response was occurring in these younger populations (Andersen & Navalta, 2004). In contrast to these earlier studies, a more recent meta analytic study (Whittington et al. 2004) and a randomized controlled clinical trial (March et al. 2004) have shown more promising results. Whittington et al. (2004) conducted a meta analysis investigating the efficacy of SSRIs relative to placebo in children aged 5-18 years. These authors, using both published and unpublished studies, concluded that Flx had a favourable risk-benefit profile. The treatment for adolescents with depression study (TADS study; March et al., 2004), the first clinical trial of Flx in the absence of pharmaceutical funding, found that the combination of Flx and cognitive behaviour therapy (CBT) was superior to either Flx alone, CBT alone, or placebo.

Given these positive clinical efficacy findings, this review now turns to the documented effects, both acute and chronic, associated with Flx use in both human and animal studies to date. This is because any decision to prescribe psychotropic drugs to a young person should involve a conscious consideration of both the possible costs and benefits of such treatments. That is, not only the immediate costs and benefits, but also the possible long-term effects of such treatments.

1.5.3 Documented Effects Following Fluoxetine Exposure: Human Studies

This section on the documented effects of Flx exposure in human studies has been separated from the animal studies below. As detailed within section 1.3.1 on adolescent animal models, there are parallels that can be drawn with regards to the neuroanatomy and neurophysiology in animals and humans. However, an animal model cannot account for the complex behaviours displayed by a human going through adolescence (Spear, 2007).

The majority of the human literature is comprised of clinical trials and case descriptions regarding individuals on Flx. The human literature is strengthened by the ability to detail complex reactions to Flx such as suicidal ideation and self harm, reactions which have not and possibly cannot be displayed in an animal model. However, the human literature is also weakened by the inability to tease apart the effects of Flx from the effects of the disease process itself, thus, the direction of causality cannot be determined. For example, increased suicidal ideation has been frequently reported as a side effect of Flx. However, this is also a common symptom of depression itself (American Psychiatric Association, 2000).

1.5.3.1 Side Effects Associated with Fluoxetine Use

In addition to the highly publicized issue of increased suicidality in adolescents consuming Flx (Cohen, 2007), there are a number of other possible side effects. The potential side effects listed on the FDA Patient Information Sheet (2006) include suicide risk, mania, weight loss, sexual dysfunction, anxiety, nervousness and sleepiness. Similar adverse consequences have been documented in placebo-controlled clinical trials, as well as additional reports of agitation, aggressiveness and hostility (March et al., 2004; Safer, 2006).

1.5.3.2 Documented Effects Following Prenatal Fluoxetine Exposure

The results of human studies of the potential teratogenic effects of prenatal Flx are very mixed. Several earlier studies found that prenatal exposure to Flx was safe for the developing foetus. However, more recent investigations have reported negative outcomes such as cardiovascular anomalies (Diav-Citrin et al., 2008). It is worth noting, however, that much of the human literature describing the prenatal effects of Flx is methodologically flawed e.g., short-term follow-up periods, maternal use of substances other then Flx such as alcohol, small sample sizes,

or analyses combining several SSRIs into one group (Gentile, 2005; Tuccori et al., 2009). These flaws limit the conclusions that can be drawn. In one of the few studies to follow up individuals exposed prenatally to Flx, Nulman et al. (2002) found that children exposed prenatally to Flx did not significantly differ from controls on measures of IQ, language development or behaviour. These authors found that the duration of depression and number of depressive episodes that their mother had experienced were negatively associated with IQ and language development, respectively.

1.5.4 Documented Effects Following Fluoxetine Exposure: Animal Studies

The following section has been divided into adult, child, and adolescent Flx exposure. In addition, where possible the sections have been split into single dose, acute effects and chronic effects. The single dose section refers to the behavioural effects following the administration of a single dose of Flx. The acute effects section refers to the effects found following the chronic administration of Flx, but when testing was conducted within at least 24 hours of the last Flx dose. The chronic effects section refers to the effects of Flx found after chronic treatment with Flx, but with some washout period between the administration of Flx and the testing.

As will become apparent, most of the animal studies of the effects of Flx exposure have been conducted using adult rats, with very few studies investigating the effects following adolescent Flx exposure. In addition, it is important to note at the outset that the vast majority of the studies investigating the effects of Flx on rats have used only male subjects. This is a key limitation within the Flx literature, especially given the known higher prevalence rates of depressive and anxiety disorders in females (Palanza, 2001).

From human clinical data we would expect a single dose of Flx to produce anxiogenic effects in rat anxiety models, consistent with reported increases in anxiety immediately upon starting Flx treatment (Silva et al., 1999). In addition, if animal studies were consistent with clinical reports, we would expect to see anxiolytic effects with chronic Flx use. As will become apparent in the sections to follow the animal and clinical literature do not always converge with regards to these findings. This has led some researchers to call into question the use of some animal models, previously developed to investigate benzodiazepine action, for the investigation of antidepressant effects (Silva et al., 1999).

1.5.4.1 Documented Effects Following Adult Fluoxetine Exposure

Single Dose

There have been numerous rodent studies of the immediate behavioural effects following a single dose of Flx. These studies have used a range of doses (1mg/kg to 20mg/kg), behavioural tests (EPM, SIT) and rat species (for example, Wistar-Kyoto, Wistar, Brown Norway, Sprague Dawley). In addition, there is a wide range of time elapsed between the Flx injection and the test, extending from 20 minutes to 24 hours. The human clinical data and the serotonin hypothesis of anxiety suggest that the immediate effect of Flx would be anxiogenic, given the rise in extracellular 5-HT levels immediately following Flx exposure (Drapier et al. 2007). The majority of these studies found that a single dose of Flx produced an anxiogenic effect (Silva et al., 1999; File, Ouagazzal, Gonzalez & Overstreet, 1999; To, Anheuer & Bagdy, 1999; Silva & Brandao, 2000; Drapier et al., 2007). However, at least one study has documented an anxiolytic effect following a single injection of Flx. For example, Griebel, Cohen, Perrault & Sanger (1999) found that a single injection of Flx (20mg/kg, IP) produced anxiolytic effects on the EPM in male Wistar- Kyoto (WKY) rats when tested 24 hours after injection. These anxiolytic effects were

not found when the animals were tested only 30 minutes after the Flx injection. Contrary to the findings of Griebel et al. (1999), Silva and Brandao (2000) administered a single injection of Flx (5.6mg/kg or 10mg/kg, IP) to adult male Wistar rats and found that when administered 30 minutes prior to testing Flx produced an anxiogenic profile on the EPM. In addition, at least one study to date has found that a single dose of Flx produced no effects on an altered version of the EPM (Jones, King & Duxon, 2002).

Acute Effects

During the past decade a number of researchers have investigated the acute behavioural effects following chronic Flx administration to adult rats, focusing predominantly on locomotor activity and emotionality. Similar to the single-dose studies outlined above, these studies have used a range of doses (1mg/kg/day to 20mg/kg/day) and rat species (for example, Wistar, WKY, Sprague-Dawley, Brown Norway). Additionally, these studies have used a wide range of treatment length, ranging from 13 days (Durand et al., 1999) to 34 days (Brenes & Fornaguera, 2009). Rats treated chronically with Flx have been found to display increased emotionality on tests such as the EPM (Silva et al., 1999; Durand et al., 1999; File et al., 1999) and the open-field (Durand et al., 1999). However, Jones et al., (2002) found that Brown Norway rats treated chronically with Flx (1, 3 or 10 mg/kg/day) via food, displayed a reduced tendency to attempt escape from a modified EPM, a behaviour which these authors regarded as anxiety-related. Rats treated chronically with Flx have also been shown to display reduced locomotor activity on the EPM (Durand et al., 1999; Griebel et al., 1999; Silva et al., 1999) and open-field (Durand et al., 1999).

Long Term Effects

Investigations of the long-term effects of adult Flx use have produced no significant findings. Wegerer et al. (1999) found that chronic oral administration of Flx (5mg/kg/day) via drinking water to adult male Wistar rats (PND 50-64) did not lead to significant alterations in the density of 5-HT transporters within the frontal cortex. Norcross et al. (2008) found that chronic Flx treatment (8-12mg/kg/day) via drinking water in adult mice (3-7 weeks) did not produce significant changes in emotion-related behaviours. Similar results were documented by Oh, Zupan, Gross & Toth, 2009).

1.5.4.2 Documented Effects Following Childhood Fluoxetine Exposure

Behavioural and neurophysiological changes have been documented following early postnatal exposure to Flx in rats. Dow-Edwards (1996) found that male, but not female, Sprague-Dawley rats treated with Flx (25mg/kg/day) from PND 11 to PND 20 showed an increased startle response in an auditory startle test when tested as adults (PND 75). Bock et al. (2005) reported increased 5-HT transporter density in the frontal cortex of adult Wistar rats (PND 90) following Flx treatment from PND 2 to PND 5 (5mg/kg/day). Ansorge, Zhou, Lira, Hen & Gingrish (2004) found decreased exploratory behaviour on the EPM and open-field of mice treated with Flx from PND 4 through to PND 21 (10mg/kg/day; IP) and tested at 12 weeks of age.

1.5.4.3 Documented Effects Following Adolescent Fluoxetine Exposure

Single Dose

There have been very few studies of the acute effects of a single dose of Flx on adolescent rats. Norrholm and Ouimet (2000) administered a group of PND 21 male Sprague-Dawley rats a single injection of Flx (5mg/kg, IP) and killed them 24 hours later. This single injection was

shown to increase the total length and number of secondary dendrites within the CA1 region of the hippocampus.

Acute Effects

Consistent with the studies reported above regarding the acute effects of adult Flx use, Oh et al. (2009) found that chronic administration (four weeks) of Flx (1.5-3 mg/kg/day, PO) produced anxiogenic effects in male juvenile mice (aged 5.5 weeks) during a novelty-induced hypophagia test, the EPM and an open-field test. It is interesting to note that the anxiogenic effects observed on the EPM and open-field test were evident in the B6 strain of mouse, but not in the Swiss Webster strain.

Long Term Effects

The long term neurophysiological effects of adolescent Flx exposure have been examined in two studies utilizing rats as subjects (Wegerer et al., 1999; Norrholm & Ouimet, 2000). Wegerer et al. (1999) found that chronic oral administration of Flx (5mg/kg/day) via drinking water to male juvenile Wistar rats (PND 25-39) led to enduring region-specific alterations in the density of 5-HT transporters. Utilising ligand-binding assays these researchers found an approximately 20% increase in the density of 5-HT transporters within the frontal cortex of rats treated during the juvenile period. It is of note that these alterations were evident within the juvenile-treated group at both PND 50 and PND 90, but were absent in rats treated with Flx at a later age (PND50-64). Norrholm and Ouimet (2000) documented an arrested dendritic spine proliferation within the CA1 region of the hippocampus, but not the dentate gyrus, in male Sprague-Dawley rats following chronic exposure to Flx (5mg/kg/day; IP) during the juvenile period (PND 21-41). It is noteworthy that this effect was observed both 24 hours and three weeks following the cessation

of Flx exposure. The authors argue that the reduced dendritic spine density may be due to the effect Flx had on either the formation or the retention of new dendritic spines.

The long term behavioural effects of chronic Flx administration have been investigated in two mouse (Norcross et al., 2008; Oh et al., 2009) and one rat study to date (LaRoche & Morgan, 2007). Neither of the investigations involving mice found significant behavioural effects following chronic Flx exposure, when tested on the open-field or EPM. Oh et al. (2009) documented an interesting finding whereby the mice treated with Flx as juveniles produced a differential response to Flx when re-exposed as adults. More specifically, the juvenile-exposed rats displayed an anxiogenic response to Flx as adults, which was in contrast to previously unexposed- adult rats who displayed an anxiolytic response to Flx.

In the only investigation of the long-term behavioural effects following adolescent Flx exposure in rats, LaRoche and Morgan (2007) documented sex-specific effects on a visual discrimination task in Long Evans rats. These authors found that females chronically treated (PND 25-49) with Flx (10mg/kg/day; PO) showed improved performance on a visual discrimination task following a 14-day washout period, as evidenced by shorter response latencies, faster reaction times and fewer omission errors. In contrast, males treated with Flx (5 or 10mg/kg/day; PO) exhibited a dose-dependent decline in performance, evidenced by decreased overall percent correct and slower reaction times on the tasks. LaRoche and Morgan (2007) also conducted an open-field test and an EPM when the rats had reached later adulthood (PND 260 and 261, respectively). Flx-treated rats did not display any significant differences during either of these behavioural tests of emotionality.

1.6 The Current Study

The current study investigated both the subsequent short-term and longer-term behavioural effects of chronic adolescent Flx exposure, utilizing a rat model. It extended the results of previous studies, and utilised both male and female subjects. In addition, the adolescent period was broken down into early and late phases in order to investigate more precisely any sensitive periods to exposure. The behavioural paradigms used for the current study allowed for an investigation of the effects of adolescent Flx exposure on later motor activity, emotionality, short-term memory and social interaction in rats.

2.0 Aims and Hypotheses

The current study aimed to extend the results of LaRoche and Morgan (2007) by treating adolescent PVG/C Hooded rats throughout the adolescent period recommended by Spear (2007), and testing the rats at two earlier intervals during adulthood (PND 70 and PND 120). More specifically, the defined 'adolescent period' was split into early (PND 28-41) and late phases (PND 42-55), so that a separate investigation of the timing of adolescence was enabled. Given that this is the first study to compare early versus late phases of adolescence, a specific hypothesis regarding which group would be most affected was not formulated.

Anxiety (or emotionality) was the targeted behaviour of the investigation, given the known actions of Flx on the serotonergic system (Silva et al., 1999), and the well- documented relationship between 5-HT and anxiety (File & Seth, 2003). We would expect acute anxiolytic effects following chronic administration of Flx, as this would be consistent with the clinical literature (Silva et al., 1999). It was also hypothesized that the long-term behavioural effects of chronic Flx administration would be anxiogenic, based on the literature documenting neurophysiological changes following adolescent Flx use (Wegerer et al., 1999; Norrholm & Ouimet, 2000).

3.0 Method

3.1 Subjects

The subjects for the present study were 40 female and 40 male PVG/C Hooded rats from the Animal Facility within the Department of Psychology, University of Canterbury, New Zealand. The rats were housed with their mothers from birth until PND 25. Following this, the rats were weaned and randomly allocated into same-sex housing pairs.

All procedures outlined below regarding housing, treatment, and testing, was approved by the Animal Ethics Committee of the University of Canterbury.

3.2 Housing

On PND 25, the same-sex housing pairs were placed within large plastic cages of the following dimensions; 410mm x 615mm x 230mm. Contained within each cage was a mesh separation, designed for the purposes of this experiment, with the aim of separating each rat within a housing pair. These mesh separations spanned the length and height of the cage, with heights corresponding to the position of the lid (120mm at the shortest point and 215mm at the highest point). The mesh itself was approximately 2mm thick. The mesh separations allowed each rat to both see and smell the other rat in the pair, and provided each rat with their own individual fluid source. Each rat had access to their own individual fluid source which consisted of a glass bottle holding approximately 500ml of either water or treatment solution (see description below). The rats remained within these sectioned cages from PND 25 until the end of the treatment phase, on PND 55.

Following the treatment phase the rats were randomly combined to create groups of 2-3 rats, of varying treatment conditions. The rats were housed within these groups in plastic cages (525mm

x 330mm x 230mm) under standard laboratory conditions. Each rat within a cage was individually identifiable through the use of non-toxic spray paint, which was administered to the upper 2cm of their tails on a fortnightly basis.

Throughout this experiment, the rats were housed within the same Holding Room in the Animal Facility at the University of Canterbury. This room was held at constant humidity (48% ± 10%) and temperature (22°C ± 2°C), and lighting was controlled by a 12-hour light/dark cycle in which the lights came on in the room at 8am. The rats had free access to both food and water (with or without Flx) throughout this experiment.

3.3 Fluoxetine Hydrochloride Treatment

The 40 female and 40 male rats were randomly allocated on PND 25 to one of four treatment conditions which spanned the adolescent period recommended by Spear (2007): early, late, early plus late, or control. Ten female and ten male rats were allocated to each of these treatment conditions. The early treatment group (E) received treatment during the period PND 28 through PND 41. The late treatment group (L) received treatment during the period PND 42 through PND 55. The early plus late group (EL) received treatment across both these intervals, from PND 28 to PND 55. The control group (C) did not receive Flx treatment.

Due to the combination of the requirements of the SIT, the paired housing and the litter sizes, two extra C and two extra E females were required. The extra two E females were used for all the procedures within this investigation, however the two extra C females were removed from the experiment following the acute open-field test (PND 55).

Flx was orally administered to the treatment group rats via drinking water. Oral administration of Flx was used in order to mimic clinical use, in which Flx is taken orally in a tablet form. Oral administration of Flx via drinking water has been effectively used in a number of previous studies using both mice (Dulawa, Holick, Gundersen & Hen, 2004; Norcross et al., 2008) and adult rats (Wegerer et al., 1999; Silva & Brandao, 2000; Thompson et al., 2004). Thompson et al. (2004) found similar serum levels of Flx and norfluoxtine following oral administration, as to those levels reported following injections or gavages. In addition, the stability of Flx in water has also been shown to be favourable for at least eight weeks (Peterson, Risley, Anderson & Hostettler, 1994). Solutions of Flx hydrochloride and water were prepared one-litre at a time within the Animal Facility. Due to the slightly different rates of water consumption per bodyweight, the concentration of the solutions differed slightly for the male and female rats. The concentrations of the solutions prepared for the males and females were 0.052mg/mL and 0.043mg/mL, respectively. These concentrations were calculated on the basis of the rates of fluid consumption and the bodyweights of a select few rats who were monitored from PND 25 through to PND 28.

These solutions were used to both fill and top-up the rat's water on a set schedule. The fluid intake of every rat (control and treatment) was monitored from PND 28 through PND 55. Fluid consumption was monitored through the weighing of the rat's water bottle every second day and water bottles were changed every four days. In addition, all rats were weighed every four days throughout the period from PND 28 to PND 55. The dose of Flx received by each individual rat was calculated on the basis of their individual fluid consumption, bodyweight and the known concentration of the solution. The range of doses is outlined within the Results section which follows.

The concentrations of Flx were made up with a target dose of 10mg/kg/day. This dose has been found to be effective in a number of previous studies and is reportedly similar to clinically used doses in humans (for example; Durand et al., 1999; Jones et al., 2002). Although this was the target dose it was anticipated that a range of doses would be found, due to individual differences in fluid consumption.

3.4 Behavioural Testing: Apparatus and Procedures

The present study involved testing of both acute and chronic effects of Flx on anxiety-like behaviour of rats. The acute phase testing was conducted on PND 55, which was the last day of treatment for both the L and EL group. The rats were removed from their Holding Room, which contained their Flx drinking bottles, and taken to the testing room. Immediately following the acute open-field test and upon return to their Holding Room, all rats were switched to untreated drinking water. It should be noted that rats from every condition were tested in the open-field during this phase for continuity of test exposure. However, the results from the early group have not been included within the statistical analysis because any effects seen in this group would not have been 'acute' effects as such because these rats had ceased treatment for fifteen days. The acute testing involved only an open-field test (detailed below).

The chronic effects of Flx were tested at both PND 70 and PND 120. The first phase of chronic effects testing was conducted on PND 70 to allow for a sufficient washout period between drug exposure and testing, ensuring that observed behavioural effects were not due to acute drug effects. Other investigators in this area have employed a washout period of this magnitude (LaRoche & Morgan, 2007). The same battery of anxiety tests was conducted on both PND 70

and PND 120, consisting of a SIT, light-dark box test and responsiveness to change in a Y-maze. The testing involved three days at both testing phases, and the sequence of test administration was the same at both PND 70 and PND 120. On the first day, the testing involved the light-dark box administered first followed by a Y-maze test. On the second day, the rats were tested utilizing the social interaction procedure. On the third and final day of testing, each rat was tested on the Y-maze on the side opposite to that which it was exposed to on day one.

All behavioural testing was conducted during the light phase of the light-dark cycle, between the hours of 0800 and 1500. In addition, all testing was conducted in the same Experimental Room, in which the temperature and humidity was held constant at 22°C ±2°C, and 48% ±10%, respectively. The level of illumination was dim and held constant at 44 lux.

All the behavioural tests employed within this investigation were non-invasive, unconditioned tests, which drew upon the rats' natural tendency to explore novel environments. The procedures utilized were absent of any food/fluid deprivation or shock treatment. Upon completion of a test, each rat was placed within a holding cage in the Experimental Room, and held within this cage until all rats from that cage had been tested. Once all the rats from that particular cage had been tested, the rats were placed back into their original cage and taken back to their Holding Room. In addition, all testing equipment was cleaned using 2% Powerquat Blue solution between animals.

3.4.1 Acute Open-Field Test

The open-field test is a popular test of anxiety-like behaviour in animals. It involves exposing an individual animal to a novel environment in which escape is not possible (Prut & Belzung,

2003). When exposed to the stress of the open-field, a range of behaviours have been consistently documented throughout the literature as being indicative of heightened anxiety. A more anxious animal will occupy the corner squares more often and the centre squares less often, display less rearing but more grooming behaviour, and also ambulate less, relative to a less anxious animal (Prut & Belzung, 2003). The procedures outlined below regarding the open-field test have been successfully employed in other research within this general area (e.g., Anderson & Hughes, 2008).

The apparatus utilized for the open-field test was a large plastic box of dimensions 600mm x 600mm. The base of the apparatus was black Perspex, divided into 16 equal sized squares (150mm x 150mm) with white numbers specifying each square. These squares defined the boundaries of four centre squares, four corner squares and the remaining outer squares. Surrounding the black base of the field were 250mm high clear Perspex walls, allowing the experimenter to see which square the rat was in at all times. The apparatus sat on a table 700mm above the ground and was illuminated by dim lighting.

The rat was placed within the centre of the open-field at the start of the test and behavioural recordings commenced when the rat had been within the apparatus for six seconds. The behaviour of each rat was observed for exactly five minutes within the field utilizing a time sampling method. The time sampling method involved recording both the location and behaviour of the rat at three second intervals, which was determined by an auditory signal from a handheld "beeper" and attached earphones. The rat was deemed to be in the square in which the majority of it's body was in at the time of the 3-second signal. The following behaviours were recorded:

1. *Rearing:* the number of times the rat reared onto its two back legs either supported or unsupported by the apparatus wall.

2. *Grooming:* the number of times the rat scratched or licked its own body. NB: a recording of grooming would be given precedence over a recording of rearing, if rearing was required in order to reach the part of the body to be groomed.

3. *Ambulation:* the number of times the rat was seen in a different square from that occupied at the end of the immediately preceding 3-second signal.

4. *Centre squares occupancy:* the number of times the rat was located within one of the four centre squares.

5. *Corner square occupancy:* the number times the rat was located within one of the four corner squares.

6. *Faecal boluses:* the number of faecal boluses left in the apparatus at the conclusion of the test.

3.4.2 Responsiveness to Brightness Change in the Y-Maze

The responsiveness to a brightness change in the Y-maze provides measures of a rat's level of anxiety, preference for novelty, as well as short-term memory (Hughes, 2004; Aitchison & Hughes, 2006). Higher anxiety can be reflected in reduced time spent in and number of entries into the novel arm of the maze, relative to less anxious animals (Aitchison & Hughes, 2006).

The apparatus utilized for this test consisted of a varnished wooden Y-maze situated upon a table 700mm above the ground with dim overhead lighting (44lux). The arms of the Y-maze were positioned at 120° and contained aluminium inserts painted either black or white. The inserts covered approximately 40cm of the 45cm long arms of the maze and covered the floor, sides and end wall of the arm. The stem of the Y-maze was 30cm long, but had a wooden insert to reduce

the stem-length to only 15cm long. The stem and arms of the Y-maze were 10cm in width and 14cm in height. The entire Y-maze apparatus was covered by a clear Perspex lid which was attached by hinges at the ends of the Y.

The Y-maze testing procedure consisted of two phases: a six-minute acquisition phase and a three-minute testing phase. During the acquisition phase, the rat was initially placed within the stem of the Y-maze and spent the allotted six minutes exploring the arms of the maze, one of which contained a black and the other a white aluminium insert. Once the six minute interval had elapsed, the rat was removed from the maze and placed within a holding cage for approximately 30 seconds. The two arm inserts were removed and replaced with clean black ones. For the testing (or retention) phase the rat was placed in the stem of the Y-maze and again allowed to freely explore both arms of the maze for the three-minute trial. During this phase, the experimenter recorded the rats' movements between the arms using a computer keyboard and specially developed computer programme. The Y-maze and inserts were cleaned with 2% Powerquat solution between each test.

Each rat completed two Y-maze tests at both PND 70 and PND 120. For one of these the white insert was on the left and for the other it was on the right. The arm in which the white insert had originally appeared was termed the 'novel' arm i.e., it involved a change from white to black. The position of the novel arm was randomly determined and differed between trials for each individual rat. The change was always from one arm white and the other black to both arms black. A change to two white arms was not included to avoid possible confounding that can arise from rats' aversion to encountering both arms white (Hughes, 2001).

The recordings made by the computer programme allowed the generation of three measures for each individual rat:

1. *The percentage of entries into the novel arm:* the total number of entries into the novel arm, divided by the total number of entries into both the novel and familiar arm.
2. *The percentage of time spent in the novel arm:* the amount of time (in seconds) spent in the novel arm, divided by the total amount of time spent in both the arms.
3. *The total entries of both arms:* the number of entries into the novel arm plus the number of entries into the familiar arm.
4. *The total time spent in both arms:* the amount of time (in seconds) spent in the novel arm plus the amount of time (in seconds) spent in the familiar arm.

3.4.3 Light-Dark Box Test

The light-dark box test provides a measure of the rat's emotional reactivity. This test draws upon the natural conflict between rats' motivation to explore novel environments and their avoidance of brightly lit areas (Hughes, Desmond & Fisher, 2004; Ohl, 2003). In the light-dark box test, an increased number of transitions between the light and dark compartments in the absence of increased general locomotor activity, is thought to reflect anxiolytic activity (Bourin & Hascoët, 2003). An increased amount of time spent in the light compartment is also thought to reflect anxiolytic effects (Bourin & Hascoët, 2003). The procedures outlined below regarding the light-dark box have been successfully employed in previous research (see Hughes, Desmond & Fisher, 2004).

The apparatus for this test was a wooden shuttle box containing two compartments of equal size (300mm long x 200mm wide x 300mm high) that were separated by a removable wooden

partition. The wooden partition covered a 100mm x 100mm doorway which, in its absence, allowed the rat free movement between the two compartments of the shuttle box. The lids covering the two compartments differed, with one side of the box containing a hinged wooden lid (the dark side) and the other side containing a clear Perspex lid (the light side).

The rat was initially placed in the dark side of the shuttle box while the wooden partition was in place, thus confining the rat to the dark compartment of the box. After approximately ten seconds, the partition was removed, exposing the rat to the light side, and enabling the rat to move between the two compartments. The rat remained within the shuttle box for five minutes and the following measures were taken using a handheld stopwatch and counter:

1. *Latency:* the time (in seconds) it took for the rat to make it's first transition into the light side. If the subject did not enter the light during the 5-minute trial, they were assigned a latency score of 300 seconds.
2. *Total time spent in the light side:* the total amount of time (in seconds) the rat spent in the light side of the apparatus.
3. *Transitions:* the total number of changes from the dark to the light side. A transition was defined as the rat placing all four paws into the light side of the box (Bourin & Hascoet, 2003).
4. *Faecal boluses:* the number of faecal boluses left in the apparatus at the conclusion of the test.

At the end of the trial the rat was removed from the apparatus and placed in the Y-maze for their acquisition trial. The light-dark shuttle box was cleaned with 2% Powerquat and dried before the next subject was tested.

3.4.4 Social Interaction Test (SIT)

The SIT is a frequently used test of anxiety in rats, which avoids the use of shocks, training or food/water deprivation (File & Seth, 2003). The SIT provides measures of social interactions, locomotion, rearing and grooming activities (Aitchison & Hughes, 2006). According to File and Seth (2006), an increase in social interaction, in the absence of increased motor activity, is indicative of an anxiolytic effect. In contrast, a decrease in social interaction is characteristic of an anxiogenic effect.

The apparatus utilised for the SIT test was similar to that outlined above for the acute open-field test. It comprised a large wooden box (600mm x 600mm) that had walls 250mm high. The inside of the apparatus was painted black and the floor was divided into 16 squares (each measuring 150mm x 150 mm) by a grid of intersecting white painted lines. These squares enabled the coding of locomotor activity. The apparatus was positioned on a 700mm high table and was illuminated by dim overhead lighting.

During the SIT, two rats from the same treatment condition and of the same sex were tested. The assignment of testing pairs for PND 70 was such that the rats tested together in the SIT were unfamiliar to each other, that is, they had never been housed together. The same pair was then re-tested at PND 120. The SIT began when both rats were facing each other within the centre of the apparatus, and their behaviour was recorded by means of an infrared camera mounted to the side of the apparatus via a wooden arm located 850mm above the base of the apparatus. At the end of the trial, both rats were removed from the apparatus and held individually in a holding cage within the experimental room until all the rats from their cage had been tested. They were then returned to their Holding Room. The apparatus was cleaned with 2% Powerquat and dried before the next pair was tested.

The videotapes were viewed by the experimenter at a later date and behaviours of each individual rat were recorded, as well as the number of social interactions between the two rats. The behaviour of one rat affects the behaviour of the other rat within the SIT, and in accordance, the pair were treated as a unit for statistical analysis. The same individual behaviours as outlined within the open-field section (above) were recorded for each individual rat (e.g. grooming, rearing, centre grid occupancy), again utilizing a time sampling method. A time sampling method was also employed for recording the number of social interactions between the pair. The time sampling method involved recording whether or not the two rats were interacting at three second intervals, which was determined by a handheld "beeper" and attached earphones. Behaviours that constituted social interaction included sniffing, lying adjacent to, following, crawling over/under, rearing adjacent to one another and mutual grooming.

4.0 Statistical Analyses

The acute effects and doses were analysed via Statview ANOVAs for the Macintosh. All behavioural data (except the acute open-field) were analysed by CLRAnova. ANOVAs were conducted for all measures and tested for effects of condition, sex, and age, as well as any interactions between these factors.

Data for two male subjects from within the control condition were lost due to video damage. Consequently, the data from these two males were not included in the SIT analysis, but the data from their light-dark box and Y-maze responses are included in all other statistical analysis.

As described in the Method section, there were four extra females (two C and two E) which were required for housing purposes to ensure continuity of the procedures. These rats were excluded from the dosage calculations due to data loss. The two extra C females were included within the acute open-field analysis. However, due to an organisational error, the two extra E females were not included in these analyses. As described in the Method section, the two extra C females were removed from the investigation following the acute open-field test, and so there was no data for these two females on the later behavioural tests. The data for the two extra E females were included within the later behavioural data.

5.0 Results

5.1 Doses

For each individual rat a range of doses was calculated on the basis of daily fluid consumption and bodyweight. These daily doses were used to calculate an average dose for each individual rat during the treatment period. These individual averages were combined in order to calculate an overall average dose for each treatment group. Average group doses are reported in Tables 1 and 2 below. It is noteworthy that upon viewing the fluid consumption across groups, the rats within the treatment groups consumed comparatively less during the treatment phase. In addition, treatment subjects tended to consume less than control subjects.

Table 1
Average Doses of Fluoxetine (mg/kg/day) and S.E.M (in Brackets) Per Group

Group	Early (n=20)	Late (n=20)	Early & Late (n=20)	F (2, 54)	p
Mean (SEM)	8.04 (0.24)	6.28 (0.16)	7.35 (0.22)	37.31	.0001

Table 2
Average Doses of Fluoxetine (mg/kg/day) and S.E.M (in Brackets) Per Sex

Sex	Male (n=30)	Female (n=30)	F (1, 54)	p
Mean (SEM)	7.88 (0.19)	6.57 (0.16)	61.15	.0001

The ANOVA revealed a significant difference between treatment groups for dose (see Table 1). The highest mean dose was for the E condition (M = 8.04 mg/kg/day) and the lowest was for the L condition (M = 6.28mg/kg/day). A statistically significant sex difference was found with males receiving, on average, higher doses than females (see Table 2). Post hoc Scheffe tests revealed that the L group received significantly lower doses than either the E ($p<.05$) or EL groups

($p<.05$). There was no statistically significant difference between the doses received by the E and the EL group.

5.2 Acute Open-Field Test

Table 3
Mean (S.E.M) and ANOVA results for the Acute Open-Field Measures (Ambulation, Centre Square Occupancy, Corner Square Occupancy, Rearing, Defecation and Grooming) For Each Treatment Group

Group	Control (n=22)	Late (n=20)	Early & Late (n=20)	F (2, 56)	p
Ambulation	55.36 (1.19)	60.20 (1.85)	60.70 (2.1)	3.03	.056
Centre Square	8.32 (0.80)	7.65 (0.83)	9.85 (0.95)	1.64	.203
Corner Square	56.68 (1.06)	55.25 (1.83)	52.00 (1.55)	2.50	.091
Rearing	29.55 (1.71)	28.75 (1.82)	28.50(1.41)	.10	.905
Boluses	.86 (0.34))	.35 (0.24)	.90 (0.44)	1.04	.360
Grooming	1.95 (0.38)	2.25 (0.42)	2.10 (0.41)	.16	.851

Table 4
Mean (S.E.M) and ANOVA results for the Acute Open-Field Measures (Ambulation, Centre Square Occupancy, Corner Square Occupancy, Rearing, Defecation and Grooming) For Each Sex

Sex	Males (n = 40)	Females (n = 42)	F (1, 56)	p
Ambulation	57.53 (1.66)	59.69 (1.24)	1.38	.246
Centre squares	8.73 (0.71)	8.47 (0.72)	.03	.854
Corner squares	54.33 (1.31)	55.06 (1.20)	2.50	.091
Rearing	27.47 (1.32)	30.34 (1.33)	2.26	.138
Boluses	1.37 (0.37)	.09 (0.09)	11.4	.001
Grooming	1.7 (0.28)	2.47 (0.35)	2.78	.101

5.2.1 Ambulation

The ANOVA failed to reveal any significant sex effects. However, there was a marginally significant treatment effect for ambulation. Post hoc t tests revealed a significant difference between the C and EL conditions [$t(40)=2.25$, $p<.05$] and between the C and L conditions [$t(40)=2.24$, $p<.05$]. More specifically, both the EL and L groups made significantly more transitions than the C group.

5.2.2 Centre Square Occupancy

There were no statistically significant treatment or sex effects for centre square occupancy.

5.2.3 Corner Square Occupancy

No main effects were significant for corner square occupancy. However there was a slight trend toward the C group occupying the corner squares the most, and the EL group the least (see Table 3). A planned comparison between the C, L and EL groups was conducted, on the basis of previously documented anxiogenic effects of acute Flx administration (for example; Silva, Alves & Santarem, 1999; Durand et al., 1999). This planned comparison revealed a statistically significant difference between the C and EL groups [$t(40)=2.53$, $p<.05$] and between the C and L groups [$t(40) = 2.24$, $p<.05$] for corner square occupancy. The C group occupied the corner square significantly more than either the L or EL groups. There was no statistically significant difference between the L and EL conditions [$t(40) = 1.36$, ns].

5.2.4 Rearing

There were no statistically significant main effects for rearing.

5.2.5 Defecation

The ANOVA revealed no significant treatment effects. There was a statistically significant sex effect for defecation, with males depositing significantly more faecal boluses than females (see Table 4).

5.2.6 Grooming

The ANOVA revealed no statistically significant main effects for grooming.

5.3 Behavioural Testing for Chronic Effects of Adolescent Fluoxetine Exposure

5.3.1 Responsiveness to Brightness Change in the Y-Maze

Table 5
Mean (S.E.M) and ANOVA results for the Brightness Change Y-Maze Measures (Percentage of Novel Arm Entries, Percentage of Time Spent in the Novel Arm, Novel Arm Entered First, Total Entries Both Arms and Time Spent in Both Arms) For Each Treatment Group

Group	Control (n=20)	Early (n=22)	Late (n=20)	Early & Late (n=20)	F (3, 74)	p
% Novel Arm Entries	57.57 (1.56)	57.47 (1.17)	58.32 (1.06)	58.70 (1.35)	.28	.837
% Time in Novel Arm	58.51 (2.45)	59.82 (1.71)	59.95 (2.05)	59.42 (2.00)	.09	.965
Novel Arm Entered First	1.48 (0.08)	1.52 (0.08)	1.52 (0.09)	1.60 (0.10)	.34	.793
Total Entries Both Arms	11.32 (0.62)	11.68 (0.82)	11.25 (0.82)	12.07 (0.91)	.24	.870
Time in Both Arms	127.71 (7.93)	135.74 (7.81)	122.72 (6.43)	129.99 (8.50)	.43	.735

Table 6
Mean (S.E.M) and ANOVA results for the Brightness Change Y-Maze Measures (Percentage of Novel Arm Entries, Percentage of Time Spent in the Novel Arm, Novel Arm Entered First, Total Entries Both Arms and Time Spent in Both Arms) For Each Sex

Sex	Male (n=40)	Female (n=42)	F (1, 74)	p
% Novel Arm Entries	56.69 (0.95)	59.24 (0.82)	4.06	.048
% Time in Novel Arm	58.67 (1.35)	60.16 (1.51)	.49	.489
Novel Arm Entered First	1.50 (0.06)	1.56 (0.06)	.40	.531
Total Entries Both Arms	10.43 (0.46)	12.69 (0.59)	8.73	.004
Time in Both Arms	126.42 (5.89)	131.85 (5.26)	.38	.539

Table 7
Mean (S.E.M) and ANOVA results for the Brightness Change Y-Maze Measures (Percentage of Novel Arm Entries, Percentage of Time Spent in the Novel Arm, Novel Arm Entered First, Total Entries Both Arms and Time Spent in Both Arms) For Each Testing Age

Testing Age	PND 70 (n=82)	PND 120 (n=82)	F (1, 74)	p
% Novel Arm Entries	57.75 (1.07)	58.25 (0.83)	.12	.732
% Time in Novel Arm	59.83 (1.54)	59.04 (1.30)	.14	.710
Novel Arm Entered First	1.48 (0.07)	1.59 (0.06)	1.30	.259
Total Entries Both Arms	10.63 (0.48)	12.54 (0.43)	16.34	.0001
Time in Both Arms	121.15 (5.32)	137.26 (3.94)	9.06	.004

5.3.1.1 Percentage of Novel Arm Entries

The ANOVA failed to reveal any significant treatment or testing age effects for the percentage of novel arm entries. There was a statistically significant sex difference (see Table 6), with females making a significantly higher percentage of novel arm entries than males.

5.3.1.2 Percentage of Time Spent in the Novel Arm

There were no statistically significant main effects for the percentage of time spent in the novel arm.

5.3.1.3 Novel Arm Entered First

The ANOVA failed to reveal any statistically significant main effects for the novel arm entered first. The analysis revealed that for all rats combined, the results differed significantly from a chance expectancy of 1 (out of 2) for first entries of the novel arm (one-sample $t[81] = 12.14$, $p < 0.0001$), and 50% for repeated entries of (one-sample $t[81] = 12.54$, $p < 0.0001$) and time spent in (one-sample $t[81] = 9.34$, $p < 0.0001$) the novel arm.

5.3.1.4 Total Entries Both Arms

There were no statistically significant treatment effects for total arm entries. The ANOVA revealed a statistically significant sex effect, with male subjects making significantly fewer arm entries than females (see Table 6). A significant testing age effect was also found, with significantly more arm entries being made at PND 120 relative to PND 70 (see Table 7).

5.3.1.5 Time Spent in Both Arms

The ANOVA failed to reveal any significant sex or treatment group effects for the amount of time spent in both arms of the y-maze. However, there was a significant testing age effect, with significantly more time being spent in both arms at PND 120, than at PND 70 (see Table 7).

5.3.2 Light-Dark Box Test

Table 8
Mean (S.E.M) and ANOVA results for the Light-Dark Box Measures (Latency, Time in Light, Transitions and Defecation) For Each Treatment Group

Group	Control (n=20)	Early (n=22)	Late (n=20)	Early & Late (n=20)	F (3, 74)	p
Latency	20.25 (7.43)	18.36 (7.27)	31.90 (11.91)	36.65 (13.81)	.70	.560
Time in Light	103.43 (7.10)	108.99 (16.99)	79.95 (8.73)	99.93 (7.82)	2.67	.054
Transitions	5.30 (0.33)	5.64 (0.39)	4.82 (0.43)	5.20 (0.38)	.75	.525
Defecation	0.45 (0.21)	0.05 (0.03)	0.40 (0.22)	0.40 (0.19)	1.20	.319

Table 9
Mean (S.E.M) and ANOVA results for the Light-Dark Box Measures (Latency, Time in Light, Transitions and Defecation) For Each Sex

Sex	Male (n=40)	Female (n=42)	F (1, 74)	p
Latency	36.69 (8.97)	16.96 (5.01)	3.48	.066
Time in Light	88.38 (5.06)	107.83 (5.70)	6.21	.015
Transitions	4.93 (0.28)	5.56 (0.26)	2.68	.106
Defecation	0.56 (0.17)	0.08 (0.05)	7.87	.006

Table 10
Mean (S.E.M) and ANOVA results for the Light-Dark Box Measures (Latency, Time in Light, Transitions and Defecation) For Each Testing Age

Testing Age	PND 70 (n=82)	PND 120 (n=82)	F (1, 74)	p
Latency	11.23 (1.52)	41.94 (9.56)	11.70	.001
Time in Light	112.46 (3.41)	84.21 (6.56)	21.14	.0000
Transitions	6.66 (0.21)	3.84 (0.27)	106.93	.0000
Defecation	0.48 (0.13)	0.16 (0.07)	7.41	.008

5.3.2.1 Latency

There were no statistically significant treatment effects for latency. For sex, there was a trend toward the males taking longer to make their initial transition into the light side, but this did not reach significance (see Table 9). A statistically significant difference was found for testing age, with the subjects taking significantly longer to make their first move into the light at PND 120, relative to PND 70 (see Table 10).

5.3.2.2 Time in Light

The ANOVA revealed a statistically significant sex effect, with females spending significantly more time in the light than males (see Table 9). There was also a significant testing age effect, with subjects spending significantly less time in the light at PND 120 relative to PND 70 (see Table 10). A statistically significant treatment effect was also found (see Table 8). Post-hoc analyses revealed statistically significant differences between the L and both the E and C groups ($p<.05$). More specifically, the L group spent significantly less time in the light than both the C and E groups.

The ANOVA also revealed a significant sex-age interaction effect ($F(1, 74) = 12.20$, $p<.001$), with males showing a greater reduction in the amount of time spent in the light from PND 70 to PND 120, relative to females. As shown in Figure 1 below, there was a significant difference for the males between PND 70 and PND 120, with the males spending significantly less time in the light at PND 120 ($p<.05$). Also shown in Figure 1 is the significant difference between the sexes at PND 120, with females spending significantly more time in the light than males at PND 120 ($p<.05$).

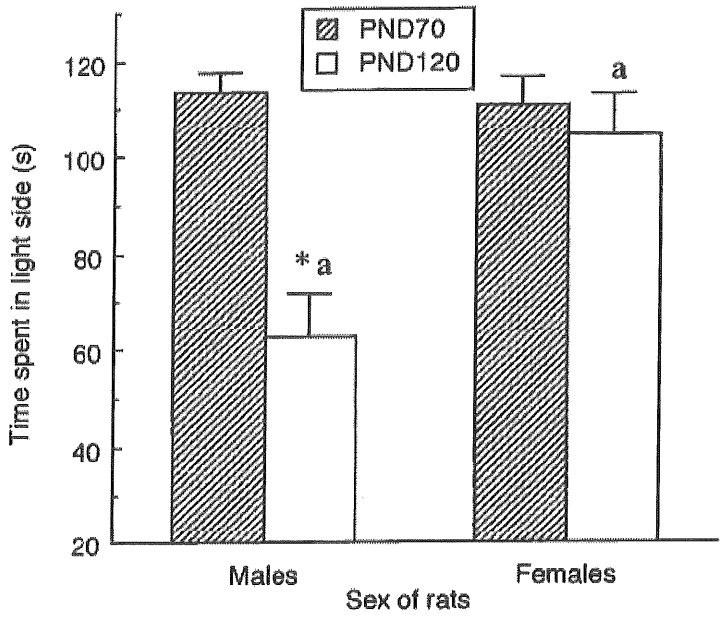

Figure 1. The mean amount of time spent in the light (in seconds) for the light-dark box test. Vertical bars denote the 0.95 confidence interval. *significantly different from PND70 for that particular sex ($p < .05$). [a]difference between the two groups indicated significant ($p < .05$).

5.3.2.3 Transitions

There were no statistically significant treatment or sex effects for the number of transitions within the light-dark box. There was, however, a statistically significant testing age effect (see Table 10) and sex-age interaction effect ($F(1, 74) = 11.00$, $p<.01$). More specifically, there was a trend toward a reduction in the number of transitions with age. This testing age effect was significant for both sexes ($p<.05$, see Figure 2). For the sex-age interaction effect, the females made a similar number of transitions to males at PND 70, but more at PND 120 (see Figure 2 below). Also shown in Figure 2 below, there was a significant difference between the males and females at PND 120, with females making significantly more transitions ($p<.05$).

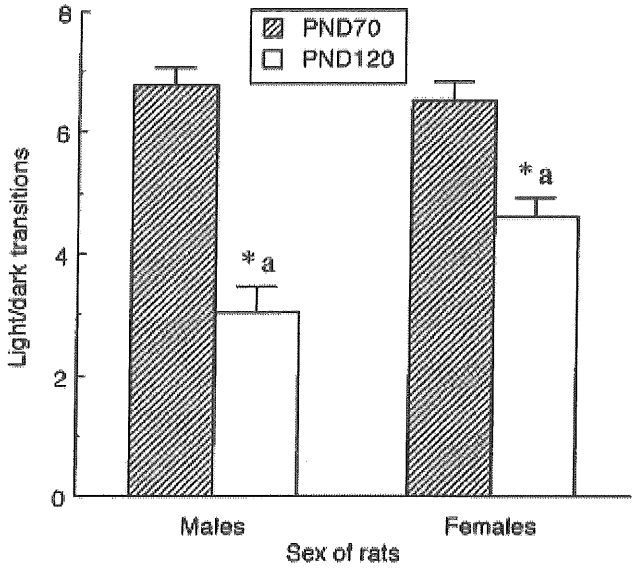

Figure 2. The mean number of light-dark transitions within the light-dark box test. Vertical bars denote the 0.95 confidence interval. *significantly different from PND70 for that particular sex ($p < .05$). [a]difference between the two groups indicated significant ($p < .05$).

5.3.2.4 Defecation

The ANOVA revealed no statistically significant treatment effects for defecation. There was a significant sex effect, with males depositing significantly more faecal boluses than females (see Table 9). However when analysed separately at each testing age, there was only a significant difference between the sexes at PND 70 ($p<.05$, see Figure 3). There was also a significant testing age effect, with significantly more faecal boluses recorded at PND 70 than at PND 120 (see Table 10). In addition, the ANOVA revealed a significant sex-age interaction ($F(1, 74) = 6.31$, $p<.05$). As can be seen in Figure 3 below, the significant sex-age effect may be due to the significant reduction in defecation by the male subjects between the two testing ages ($p<.05$).

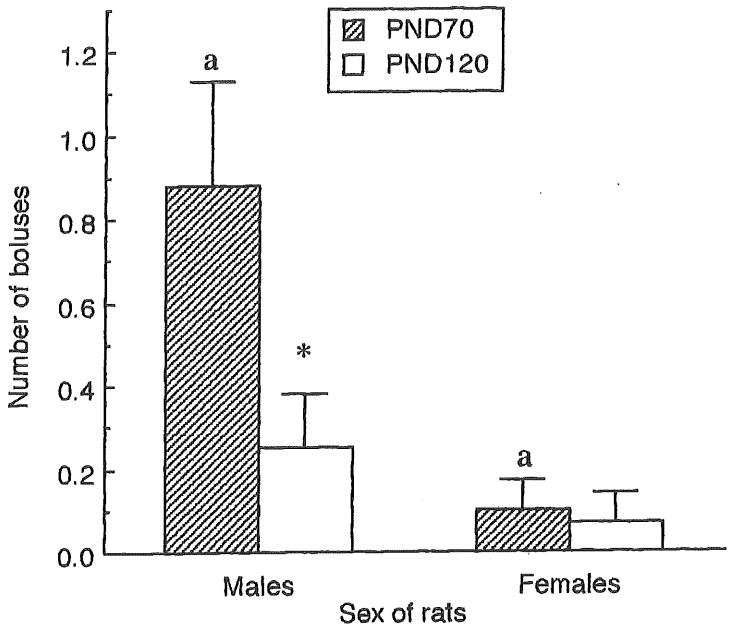

Figure 3. The mean number of faecal boluses deposited within the light-dark box. Vertical bars denote the 0.95 confidence interval. *significantly different from PND70 for that particular sex ($p < .05$). [a]difference between the two groups indicated significant ($p < .05$).

5.3.3 Social Interaction Test

Table 11

Mean (S.E.M) and ANOVA results for the Social Interaction Test Measures (Ambulation, Centre Square Occupancy, Corner Square Occupancy, Rearing, Defecation, Grooming and Social Interaction) For Each Treatment Group

Group	Control (n=18)	Early (n=22)	Late (n=20)	Early & Late (n=20)	F (3, 72)	p
Ambulation	57.03 (1.82)	56.66 (2.04)	54.47 (1.53)	56.97 (1.65)	.79	.503
Centre Square	5.53 (0.56)	4.93 (0.55)	4.88 (0.88)	4.75 (0.95)	.96	.415
Corner Square	48.53 (1.48)	47.70 (1.56)	50.80 (2.11)	47.33 (1.26)	1.65	.186
Rearing	43.64 (1.36)	40.64 (1.76)	39.90 (3.16)	42.00 (2.16)	1.15	.335
Grooming	1.33 (0.20)	1.86 (0.65)	1.27 (0.38)	1.55 (0.39)	.83	.480
Defecation	0 (0)	0.59 (0.02)	1.25 (0.47)	0.35 (0.01)	4.683	.005
Social Interactio	52.33 (2.89)	49.45 (1.97)	50.05 (2.48)	47.10 (2.40)	.630	.601

Table 12
Mean (S.E.M) and ANOVA results for the Social Interaction Test Measures (Ambulation, Centre Square Occupancy, Corner Square Occupancy, Rearing, Defecation, Grooming and Social Interaction) For Each Sex

Sex	Male (n=38)	Female (n=42)	F (1, 72)	p
Ambulation	51.49 (0.78)	60.61 (0.90)	57.00	.0000
Centre Square	4.80 (0.35)	5.19 (0.40)	.16	.695
Corner Square	51.05 (0.91)	46.27 (0.90)	14.63	.0003
Rearing	41.88 (1.03)	41.10 (1.11)	.30	.585
Grooming	1.36 (0.15)	1.67 (0.23)	1.11	.295
Defecation	1.00 (0.24)	0.17 (0.12)	10.02	.002
Social Interaction	50.07 (1.96)	49.21 (1.34)	.032	.860

Table 13
Mean (S.E.M) and ANOVA results for the Social Interaction Test Measures (Ambulation, Centre Square Occupancy, Corner Square Occupancy, Rearing, Defecation, Grooming and Social Interaction) For Each Testing Age

Testing Age	PND 70 (n=80)	PND 120 (n=80)	F (1, 72)	p
Ambulation	59.58 (0.89)	52.97 (0.98)	45.07	.0000
Centre Square	6.16 (0.41)	3.85 (0.36)	22.84	.0000
Corner Square	47.65 (1.02)	49.49 (0.90)	1.77	.188
Rearing	36.08 (0.94)	46.86 (0.88)	112.28	.0000
Grooming	1.71 (0.19)	1.33 (0.20)	2.56	.114
Defecation	0.82 (0.20)	0.30 (0.12)	10.50	.002
Social Interaction	55.65 (1.58)	43.67 (1.29)	50.68	.0000

5.3.3.1 Ambulation

The ANOVA revealed no statistically significant treatment effects for ambulation. There was a statistically significant sex effect, with females making significantly more transitions than males (see Table 12). This sex effect was statistically significant at both testing ages ($p<.05$). The

ANOVA also revealed a significant testing age effect, with greater ambulation recorded at PND 70 relative to PND120 (see Table 13). This testing age effect was significant for both sexes ($p<.05$). A statistically significant sex-age interaction effect was also revealed ($F(1, 72) = 4.85$, $p<.05$), with males rates of ambulation decreasing from PND 70 to PND120 at a greater rate than females.

5.3.3.2 Centre Square Occupancy

There were no statistically significant sex or treatment effects, although there was a significant testing age effect for centre square occupancy (see Table 13). The frequency of centre square occupancy was significantly greater at PND 70 relative to PND 120.

There were also three statistically significant interaction effects; sex-group ($F(3, 72) = 2.92$, $p<.05$), sex-age ($F(1, 72) = 4.00$, $p<.05$) and group-age ($F(3, 72) = 4.79$, $p<.01$).

For the sex-group interaction, females occupied the centre squares more often than males in both the L and EL groups. However, the males occupied the centre square more often than females in the E group. The males and females within the C group had similar rates of centre square occupancy. As shown in Figure 4 below, there was a significant difference between males within the E group and the males in both the L and EL groups ($p<.05$). More specifically, the E group males occupied the centre squares more often that the males in the L or EL groups. In addition, the rate of centre square occupancy by the males within the EL group was significantly different from the rate of centre square occupancy of the males within the C group ($p<.05$), with the EL males showing lower rates of centre square occupancy. The difference between the sexes within the E condition was statistically significant ($p<.05$).

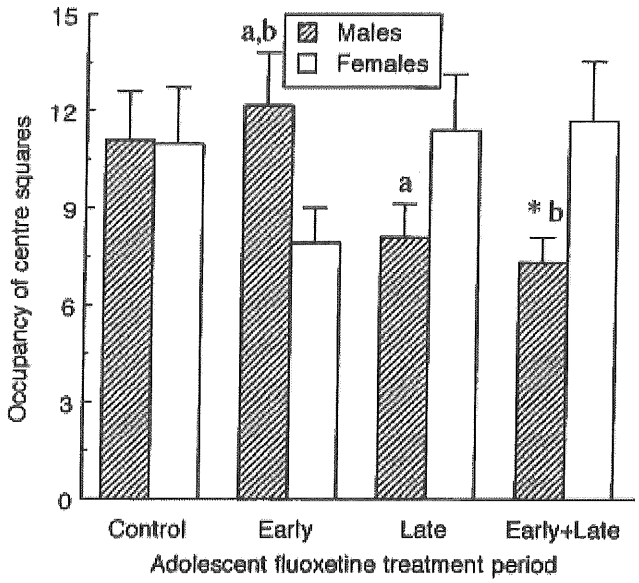

Figure 4. The mean amount of centre square occupancy within the social interaction test. Vertical bars denote the 0.95 confidence interval. *significantly different from control group for that particular sex ($p<.05$). ^{a,b}difference between the two groups with superscripts in common significant ($p<.05$).

For the sex-age interaction, at PND 70 the females occupied the centre squares more than the males, but the reverse was true at PND 120, with male subjects more often occupying the centre squares. The difference in centre square occupancy for females between the two testing ages was statistically significant ($F(1, 72) = 24.39$, $p<.001$), while the difference for the male subjects approached significance ($F(1, 72) = 3.71$, $p=.058$).

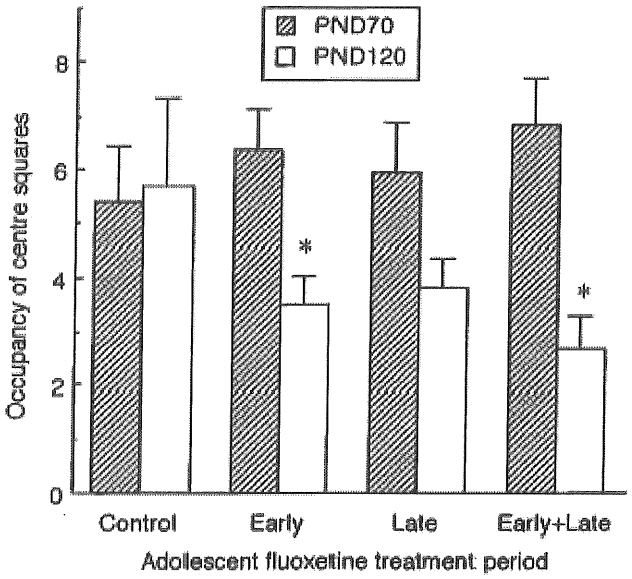

Figure 5. The mean amount of centre square occupancy within the social interaction test. Vertical bars denote the 0.95 confidence interval. *significantly different from PND70 for that particular testing age ($p < .05$).

For the group-age interaction effect, upon viewing the interaction graph (see Figure 5 above) it seems that the C group centre square occupancy increased slightly across the testing ages, however the centre square occupancy decreased across the testing ages for the treatment conditions. There were no significant differences between the treatment groups at PND 70. At PND 120, however, there was a statistically significant difference between the treatment groups ($F (3, 144) = 4.96$, $p<.01$). As shown in Figure 5 above, for both the L and EL groups there were significant differences between the centre square occupancy at PND 70 and PND 120 ($p<.05$).

5.3.3.3 Corner Square Occupancy

The ANOVA revealed no significant treatment or testing age effects for corner square occupancy (see Tables 11 and 13). There was a significant sex effect, with males occupying the corner square significantly more than females (see Table 12). There was also a statistically significant sex-treatment interaction ($F (3, 72) = 2.85$, $p<.05$). For the males there was a statistically

significant difference between the E and L groups, with the L group spending significantly more time occupying the corner squares ($p<.05$, see Figure 6 below). Post-hoc analyses revealed a significant difference between the male EL group and the female EL group ($p<.05$), with males occupying the corner squares more often. A similar result was found for the L groups, with the males in the L group occupying the corners significantly more than females in the L group ($p<.05$).

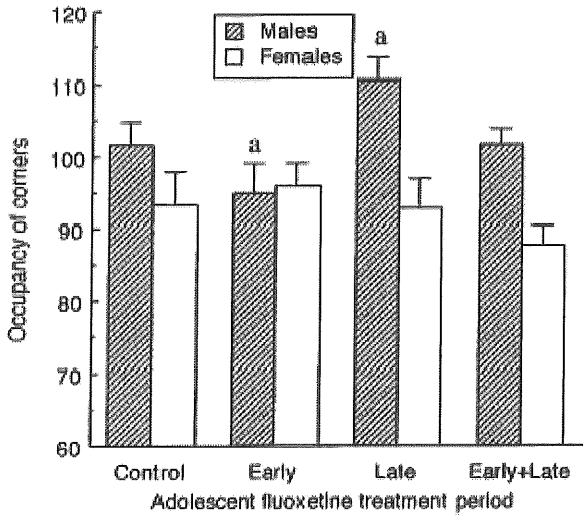

Figure 6. The mean amount of corner square occupancy within the social interaction test. Vertical bars denote the 0.95 confidence interval. [a]difference between the two groups indicated significant ($p <.05$).

5.3.3.4 Rearing

There were no statistically significant group or sex effects for rearing. There was a statistically significant effect for testing age, with subjects engaging in significantly more rearing at PND 120 than PND 70 (see Table 13).

5.3.3.5 Grooming

The ANOVA revealed no statistically significant main effects for grooming.

5.3.3.6 Defecation

The ANOVA revealed a statistically significant treatment effect, with the L group depositing the highest number of faecal boluses and the C group depositing the least (see Table 11). The ANOVA also revealed a significant sex effect, with males defecating significantly more than females (see Table 12). There was also a significant age effect, with less defecation at PND 120 relative to PND 70 (see Table 13).

There was also a significant group-age interaction ($F(3, 72) = 4.22$, $p<.01$). When analysed separately, there was a statistically significant group effect at PND 70 ($p<.05$), but not at PND 120. At PND 70, the L group had significantly higher rates of defecation relative to the other groups ($p<.05$, see Figure 7 below). In addition, there was a statistically significant difference within the L group regarding the amount of defecation between PND 70 and PND 120 ($p<.05$).

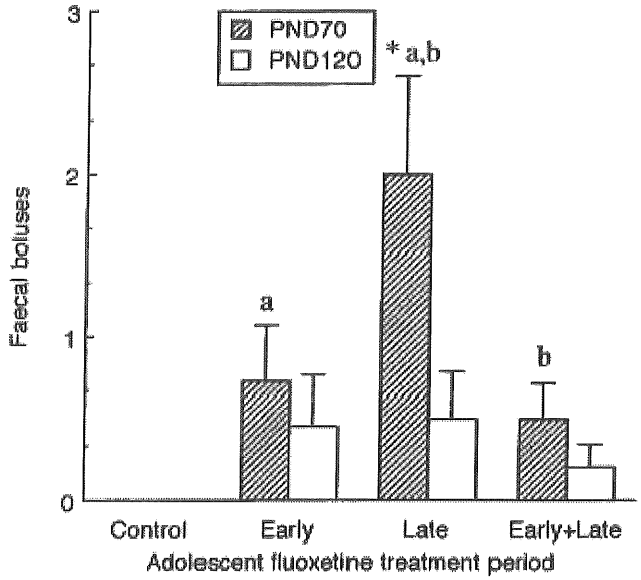

Figure 7. The mean number of faecal boluses within the social interaction test. Vertical bars denote the 0.95 confidence interval. *significantly different from PND120 for that particular sex ($p < .05$). ^{a,b}difference between the two groups with superscripts in common significant ($p < .05$).

5.3.3.7 Social Interaction

There were no statistically significant sex or treatment effects for social interaction. There was a statistically significant effect for testing age (see Table 13), with higher rates of social interaction at PND 70 relative to PND 120.

Table14

Summary of the sex, testing age and treatment effects for all the behavioural tests.

Sex Effects		
Acute Open-field		
	Defecation: males > females	
Y-maze		
	Percentage of novel arm entries: females > males	
	Total arm entries: females > males	
Light-Dark Box		
	Time in light: females > males $*^{120}$	
	Defecation: males > females $*^{70}$	
	Transitions: males < females $*^{120}$	
Social Interaction Test		
	Ambulation: females > males	
	Corner squares: males > females	
	Defecation: males > females	
Testing Age Effects		
Acute Open-field		
	Not relevant	
Y-maze		
	Total arm entries: PND 120 > PND 70	
	Time spent in both arms: PND 120 > 70	
Light-Dark Box		
	Latency: PND 120 > PND 70	
	Time in light: PND 70 > PND 120 *males	
	Defecation: PND 70 > PND 120	
	Transitions: PND 70 > PND 120	
Social Interaction Test		
	Defecation: PND 70 > PND 120	
	Rearing: PND 120 > PND 70	
	Ambulation: PND 70 > PND 120	
	Centre squares: PND 70 > PND 120 *females	
	Interactions: PND 70 > PND 120	
Treatment Effects		
Acute Open-field		
	Ambulation: EL, L > C	
	Corner squares: L, El < C	
Y-maze		
	No significant results	
Light-Dark Box		
	Time in light: C, E > L	
Social Interaction Test		
	Centre squares:	males E > males L, EL
		males EL < males C
		E group: M > F
		L group: PND 70 > PND 120
		EL group: PND 70 > PND 120
	Corner squares:	males E < males L
		EL group: F < M
		L group: F < M
	Defecation:	L > EL, E, C $*^{120}$
		L group: PND 70 > PND 120

$*^{120}$ denotes significance ($p<.05$) for PND 120 only; $*^{70}$ denotes significance ($p<.05$) for PND 70 only; *females denotes significance ($p<.05$) for females only; *males denotes significance for males only.

6.0 Discussion of Results

The current study randomly assigned male and female adolescent PVG/C Hooded rats to one of three Flx treatment conditions or a control group (C): early (E; PND 28-41), late (L; PND 42-55) or early plus late (EL; PND 28-55). The individual consumption and weights of all rats was monitored from PND 28 through to PND 55. The control group received water throughout this period, while the treatment groups received Flx dissolved in their drinking water for their treatment period (denoted in brackets above) and water outside this period. All the subjects were tested on an acute open-field test on PND 55, when the L and EL groups were still receiving treatment. At PND 70 and PND 120 the rats were tested on three measures of emotionality: light-dark box, responsiveness to brightness change Y-maze and the SIT.

6.1 Doses

For all treatment groups, the doses received were below the target of 10mg/kg/day. There were significant differences between groups for the doses received, with the L group receiving significantly lower doses of Flx than either the E or EL groups. In addition, males received higher doses of Flx than the females across groups.

The lower doses are a reflection of reduced fluid consumption by the rats during their treatment phase. Reduced fluid intake during Flx treatment via drinking water has been previously documented using male Wistar rats (Silva & Brandao, 2000; Thompson et al., 2004), indicating that this finding is not specific to the current study. The reduced fluid intake likely indicates a slight aversion by the rats to the taste of Flx. Prendergast, Hendricks, Yells and Balogh (1996) documented a conditioned taste aversion toward a sucrose solution when rats were pre-treated with Flx. The reduced fluid intake may also be related to the anorexic effects of Flx. Thompson et al. (2004) noted that rats are prandial drinkers, indicating that they tend to consume fluid when they eat, and so a reduction in food intake

may also lead to a reduction in fluid intake. In the current study, the food intake was not monitored. However, the bodyweights of Flx-treated and control rats were similar post-treatment.

Although the doses within the current study were below the targeted 10mg/kg/day, the average doses for each group fell within a dose range that has been documented within the literature as showing significant behavioural (Drapier et al., 2007; Silva et al., 1999; Jones et al., 2002) and neurophysiological (Norrholm & Ouimet, 2000; Wegerer et al., 1999) effects. For instance, Drapier et al. (2007) found significant behavioural changes on the EPM for rats treated with a single dose of 5mg/kg Flx. It is noteworthy that on most measures within the study by Drapier et al. (2007), the 5mg/kg group displayed similar results to those receiving a dose of 10mg/kg.

Although the three Flx treatment groups experienced doses which differed significantly from one another at a statistical level, it seems unlikely that these differences were pharmacologically significant. As noted above, the rats within the L group received the lowest average dose of Flx, but it was these rats which displayed the most emotionally reactive profile later on, suggesting that these rats were the most affected by Flx treatment (see section 6.2 Treatment Group Effects below).

6.2 Treatment Group Effects

This section has been broken down into the acute and chronic effects following Flx administration. For the current study, acute effects refer to those behavioural effects found on the acute open-field test. That is, effects observed immediately following the cessation of treatment. The chronic effects of Flx in the current study refer to effects observed after the 15-day washout period. That is, effects observed during behavioural testing on PND 70 and PND 120, which consisted of a light-dark box test, responsiveness to change in a Y-maze and a SIT.

6.2.1 Acute Effects

The results of the acute open-field test suggest that Flx had an anxiolytic effect following chronic Flx treatment. During the acute open-field test, the groups actively receiving Flx treatment at the time of testing (EL and L) displayed significantly increased rates of ambulation and possibly reduced corner square occupancy, relative to control subjects. These findings suggest decreased emotional reactivity and increased locomotor activity for these treatment groups.

Evidence of decreased emotionality is consistent with clinical reports and at least one other investigation involving chronic Flx treatment in rats (Jones et al., 2002) and one involving mice (Dulawa et al., 2004). Clinical reports have described the effectiveness of chronic Flx in alleviating anxious and depressive symptomology (Whittington et al., 2004). On their modified version of the EPM, the unstable elevated exposed plus maze (UEEPM), Jones et al. (2002) found that male Brown Norway rats chronically treated (21 days) with Flx (1, 3 or 10 mg/kg/day) via food, displayed an anxiolytic profile 24 hours after their last exposure. Flx reduced the rats' tendency to escape and decreased escape-related behaviours within the UEEPM, as indicated by decreased escape rates, increased trial duration, decreased attempts to jump off or turn toward the end of the apparatus. Dulawa et al. (2004) found that chronic (24 days) Flx treatment (18mg/kg/day) administered via drinking water to BALB/c mice led to anxiolytic effects when tested in the open-field test and a novelty-induced hypophagia task.

The results of the current study are, however, inconsistent with the results of several other investigations of the acute effects following chronic Flx administration in adult rats. There has been a trend within these particular studies for adult rats treated chronically with Flx to display anxiogenic activity when tested a short period (up to 48 hours) after their Flx exposure. A variety

of rat strains treated chronically with Flx have been found to display increased emotionality on tests such as the EPM (Silva et al., 1999; Durand et al., 1999; File et al., 1999) and the open-field test (Durand et al., 1999). For example, following chronic administration (22 days) of Flx (5mg/kg; IP), adult male Wistar rats displayed an anxiogenic profile on the EPM when subjects were tested 60 minutes after their last injection, making fewer entries into and spending less time in the open arm of the EPM (Silva et al., 1999).

Evidence of increased locomotor activity following chronic Flx administration in the current study also contradicts the results of several other investigations of the immediate effects of chronic Flx administration in adult rats. The literature to date using adult rat subjects documents either no effect on locomotor activity (Brenes & Fornaguera, 2009; Durand et al., 1999; File et al., 1999; Griebel et al., 1999; Jones et al., 2002; Silva & Brandao, 2000; Thompson et al., 2004; To et al., 1999) or a reduction in locomotor activity (Durand et al., 1999; Griebel et al., 1999; Silva et al., 1999) shortly following chronic Flx administration. Even when tested in an open-field, the same measure as used in the current study, chronic (34 days) Flx treatment (10 mg/kg/day) did not significantly affect the ambulation of adult Sprague-Dawley rats when tested during the dark phase, 10 hours after their last dose of the drug (Brenes & Fornaguera, 2009). Durand et al. (1999) conducted an acute open-field test during the light phase of SHR and WKY rats exposed to 5 or 10 mg/kg/day of Flx (IP). These authors found that locomotor activity was unchanged in the SHR rats following Flx treatment, but decreased in Flx treated WKY rats at both the 5 and 10 mg/kg/day doses.

The current study seems to be the first to show increased ambulation on the open-field test immediately following chronic administration of Flx in rats, although one study using mice

documented increased locomotor activity following Flx treatment in one strain (Dulawa et al., 2004). Dulawa et al. (2004) compared the effects of Flx on four male mouse strains on the open-field test: BALB/c, DBA/2, C57BL/6 and 129SvEv. For two of the mouse strains (C57BL/6 and 129SvEv) Flx treatment decreased locomotor activity, for another strain locomotor activity increased following Flx treatment (DBA/2), while yet another mouse strain displayed no significant changes in locomotor activity (BALB/c) following Flx treatment. This study, although using mouse subjects, highlights the importance of strain/species for the effects of a drug.

There are three key differences between the subjects used in the current study and those used within the chronic Flx investigations cited above. The current study used both female and male subjects, the subjects were PVG/C Hooded rats and they were in the late phase of adolescence during the acute open-field testing. It is a common finding within the open-field literature that females have higher motor activity levels than males (for example; Broadhurst, 1958). When viewing the acute open-field data, the ambulation scores were higher for the females, but this did not reach statistical significance, and there were no significant sex-treatment interactions. In addition, the trends in the male and female data were the same, with lower ambulation scores for the C group and higher scores for the L and El groups. Therefore, the findings cannot be attributed to the inclusion of female subjects.

The current study appears to be one of the first studies to use PVG/C Hooded rats to investigate the effects of Flx. The importance of strain factors in the investigation of antidepressant effects has previously been documented (Thompson et al., 2004). In addition, an early strain comparison study showed that PVG/C Hooded rats have lower levels of emotionality and ambulation relative

to albino rats when tested as adults on the open-field test (Broadhurst, 1958). Accordingly, in the current study the baseline level of ambulation and anxiety may have been lower than in previous studies, and so more opportunity may have been apparent for the acute Flx treatment to increase ambulation.

The main difference between the current study and those cited above is the age of the subjects. In the current study, the rats were in the late phase of adolescence during Flx treatment and the acute open-field test (PND 55), in contrast to the studies discussed above in which the rats were equivalent to adults. To date, there does not appear to be any other studies which have investigated the acute behavioural effects of chronic Flx administration during adolescence in rats. Although there has been at least one study using juvenile mice as subjects, these investigators found that chronically Flx- treated Swiss-Webster and C57BI/6 mice displayed an anxiogenic profile on the open-field and EPM upon acute testing (Oh et al., 2009).

6.2.2 Chronic Effects

Subjects treated with Flx during the late phase of adolescence seem to be the most affected in the long term. During the chronic effects testing, the L group displayed behaviours suggestive of heightened emotional reactivity relative to the E or C group. On the light-dark box test, subjects in the L group spent less time in the light than the C or E subjects. During the SIT, the L group defecated more than any of the other groups. In addition, the males within the L group spent significantly more time within the corner squares and less time within the centre squares of the SIT apparatus than males from the E group. The EL group also displayed signs of heightened emotionality, with males from the EL group occupying the centre squares of the SIT significantly less than the males within the C group.

The current results suggest that the length of Flx treatment during adolescence is not an important determinant of the long-term sequelae, but, what is important, is timing. More specifically, whether the subject was treated during the late phase of adolescence. During the chronic effects testing, the only statistically significant difference between the EL and the L group was for defecation in the open-field at PND 70, with the L group defecating significantly more than the EL group. When this result is considered with reference to the other results in this study showing the similar effects found between the EL and L groups, it seems reasonable to conclude that both the EL and L groups were more affected than the E group by Flx exposure, and these groups were affected in ways which increased their emotional reactivity.

The statistically significant differences in the doses received by the L group and both the E and EL group did not appear to translate into pharmacologically significant differences. As noted above, the E and EL groups received significantly higher doses of Flx than the L group, and so we may expect to observe a dose-response trend with the two former groups displaying more drastic changes during behavioural testing relative to controls. This is most certainly not the case, as the L group appears to be the most affected in the behavioural testing with regard to emotional reactivity.

The current study found no significant effects of Flx on short term memory. This is evidenced by the lack of significant differences exhibited between the control and treatment groups on the Y-maze. This result is surprising given the documented effects of Flx on the hippocampus, which plays a crucial role in memory (Norrholm & Ouimet, 2000).

The results of the current study showing significant behavioural changes following chronic Flx treatment during adolescence are inconsistent with other rat (LaRoche & Morgan, 2007) and mouse (Norcross et al., 2008; Oh et al., 2009) studies documenting no significant long term changes in emotional reactivity following chronic Flx use. For example, Oh et al., (2009) found that chronic (4 weeks) Flx exposure (1.5-3 mg/kg/day) had no significant long-term effects on Swiss-Webster or C57B1/6 mice on the EPM or open-field.

The only other study using rat subjects to investigate the long term behavioural effects of adolescent Flx exposure was conducted by LaRoche and Morgan (2007). These authors chronically treated male and female Long Evans rats with Flx (5, 10 or 15mg/kg/day; PO) from PND 25 through to PND 49. An open-field and EPM test were conducted during later adulthood (PND 260-261) and the authors found no significant differences between the anxiety-like behaviour of the control versus the Flx rats. The lack of significant findings by LaRoche & Morgan (2007) are in contrast to the current study which found long-term anxiogenic effects of Flx exposure.

Several reasons are possible for the different results of the current study from those of LaRoche and Morgan (2007). First, as described above, the treatment of rats during the late adolescent phase seems to be the crucial determinant of long-term effects. LaRoche and Morgan (2007) treated their subjects from PND 25 through to PND 49, which overlaps more with the early than the late treatment phase of the current study. No significant changes were observed for the E group in the current study, so we could say that the treatment group which overlapped the most with that of LaRoche and Morgan (2007) was consistent with their results. Secondly, we always need to consider the possibility that the results found in the current study are strain-specific

(Thompson et al., 2004). The current study used PVG/C Hooded rats, while LaRoche and Morgan (2007) used Long Evans rats. As such, the differential findings between the current study and that of LaRoche and Morgan (2007) may reflect strain differences in the timing of adolescence or the sensitivity of the serotonergic system. Thirdly, the light-dark box and the SIT may be more sensitive to the long-term effects of Flx than the open-field or EPM. The use of the open-field in the investigation of the effects of antidepressant medications has been previously criticized (Prut & Belzung, 2003). Fourth, the behavioural testing in the LaRoche and Morgan (2007) study was conducted in very late adulthood (PND 260-261), in contrast to the current study which conducted behavioural testing at human-equivalent of early and middle adulthood (PND 70 and PND 120, respectively). It may be that there is a period in early-middle adulthood in which the long-term effects of Flx are evident, but in later adulthood these effects dissipate as baseline anxiety levels increase (Boguszewski & Zagrodzka, 2002).

It is possible that during the testing at PND 70, both the EL and L groups were withdrawing from Flx use. As outlined by Julien (2001) the main psychological symptoms associated with the serotonin withdrawal syndrome in humans include anxiety, agitation, crying spells and irritability. Julien (2001) also notes other symptoms that occur less frequently such as overactivity and memory problems. However, the washout period adopted in this study was consistent with at least one other study (LaRoche & Morgan, 2007).

6.3 Testing Age Effects

The rats tended to be more emotionally reactive and display less motor activity at PND 120 than at PND 70. Heightened anxiety at PND 120 was evidenced by longer latencies to enter and less time spent within the light side of the light-dark box. The reduction in motor activity was

evidenced by the decreased number of transitions at PND 120 within both the light-dark box and the SIT. Within the SIT, higher rates of social interaction and centre square occupancy were found at PND 70 relative to PND 120.

In contrast to the above findings, the frequency of rearing was significantly higher at PND 120 than PND 70 within the SIT. In addition, higher rates of defecation, a proposed measure of emotional reactivity in rats, were found at PND 70 within both the light-dark box and the SIT. Although generally regarded as a rough measure of emotionality, this interpretation has been criticised (Johnston & File, 1991). Some authors have suggested that factors such as body weight or food intake could account for defecation differences (Johnston & File, 1991). However, data to support these speculations is limited.

In the Y-maze, rats spent more time in both arms of the maze and also had a higher total number of both arm entries at PND 120, relative to their performance at PND 70. This result may also be explained by heightened emotionality at PND 120. During the y-maze, the experimenter was located adjacent to the stem of the y-maze, and so the rats may have entered the arms and spent more time in the arms in an attempt to avoid the experimenter.

The results of the current study are consistent with the literature to date documenting increased anxiety, decreased motor activity, and decreased social interaction with age (Boguszewski & Zagrodzka, 2002; Salchner, Lubec & Singwald, 2004). For example, Boguszewski and Zagrodzka (2002) showed that in the EPM, SIT and open-field, older rats (aged 24 months) were more anxious and displayed less motor activity than younger adult rats (aged 4 months).

6.4 Sex Effects

Significant sex differences were documented for all behavioural tests: acute open-field, light-dark box, Y-maze and the SIT. On the acute open-field test, the only significant sex effect was for defecation, with males depositing more faecal boluses than females. This higher rate of defecation for males was found not only on the acute open-field, but also during the chronic effects testing in the SIT and the light-dark box. Higher defection rates by males rats have been found consistently throughout the literature (Broadhurst, 1958; Johnston & File, 1991; Palanza, 2001).

During the chronic effects testing, the males tended to display higher levels of anxiety than females. This was evident in the light-dark box results, with males spending significantly less time in the light side of the apparatus during PND 120 testing. Heightened emotionality in males was also evident for the SIT results, whereby males spent more time in the corner squares. These findings are consistent with abundant literature reporting higher anxiety levels in male rats relative to females (e.g., Palanza, 2001; Hughes, Desmond & Fisher, 2004).

For all tests, females showed higher levels of motor activity than males. This was evidenced by a higher total number of arm entries in the Y-maze and a greater number of transitions in both the SIT and light-dark box for females. The higher activity in females is an expected finding which has been documented throughout the literature, for tests such as the open-field (Broadhurst, 1958; LaRoche & Morgan, 2007), the SIT (Johnston & File, 1991), the light-dark box (Hughes, Desmond & Fisher, 2004) and the Y-maze (Aitchison & Hughes, 2006; Anderson & Hughes, 2008; Hughes & Neeson, 2003).

In the SIT, the rate of locomotor activity decreased at a greater rate for males across testing ages. This greater reduction in locomotor activity for males across the life span has been previously documented, and reportedly occurs following the periadolescent period (Andersen, 2003). Andersen (2003) described that within the open-field, the activity levels of males reduced by half, while females' activity level only reduced by 10% following the periadolescent period. These sex differences are thought to reflect the effects of gonadal hormone exposure around the time of puberty.

In the Y-maze, female subjects made a higher percentage of novel arm entries than males. This result may reflect a greater preference for novelty in females, or again reflect their lower anxiety levels relative to male rats. The higher percentage of novel entries made in the Y-maze by females is in contrast to at least one previous study which found that males made a higher percentage of novel arm entries (Hughes, 2001), and three studies which found no significant sex differences on this measure (Aitchison & Hughes, 2006; Anderson & Hughes, 2008; Hughes & Neeson, 2003). It is possible that the increased novel arm entries resulted from superior visual attention in the female rats (LaRoche & Morgan, 2007). This would be consistent with the previously documented improved performance by Flx-treated rats on a visual discrimination task (LaRoche & Morgan, 2007).

Sex differences in response to Flx have been previously documented following adolescent (LaRoche & Morgan, 2007) and early postnatal Flx exposure (Dow-Edwards, 1996). LaRoche and Morgan (2007) documented sex-specific effects on a visual discrimination task following chronic adolescent Flx use. More specifically, females displayed improved performance following Flx exposure, while male performance on the task was impaired. Dow-Edwards (1996)

found that male, but not female, rats treated with Flx from PND 11-20 showed an increased startle response on an auditory startle test when tested at adults (PND 75).

There are a couple of possible reasons for the sex differences in the current study. First, the increased emotionality in males may reflect the higher doses of Flx they received. However, even males in the control group displayed higher emotionality. Secondly, and more likely, the sex differences may reflect a difference in the sensitivity of the male central nervous system to Flx, possibly related to gonadal hormone influences.

A relationship between 5-HT, estrogen and mood regulation has previously been described in both human and animal studies (Rubinow, Schmidt & Roca, 1998). Estrogen refers to a class of sex hormones which are implicated in the development of female sexual organs, thus, are found in higher levels in females relative to males. Rubinow et al. (1998) suggested that one of these hormones, estradiol, may regulate 5-HT through actions on the 5-HT receptor number and function. These authors described that the actions of estradiol on 5-HT may also play a modulatory role in the effects of 5-HT acting drugs. In accordance, the effects of Flx may be modulated by estradiol in females, an effect which would likely be less evident in males.

6.5 Methodological Limitations

One of the main limitations of the current study was the variation in the doses received both across sexes and treatment groups. This variation and possible reasons for this was explained in section 6.1 above. Although the group doses were significantly different at a statistical level, it is unlikely that the difference in doses was pharmacologically significant, with less than 2mg/kg/day separating the average doses for the groups. In addition, although the average doses were below the planned

10mg/kg/day, they were still all above 5mg/kg/day, a dose for which significant behavioural (Drapier et al., 2007; Silva et al., 1999; Jones et al., 2002) and neurophysiological (Norrholm & Ouimet, 2000; Wegerer et al., 1999) effects have been documented. In addition, it is clear that the doses were pharmacologically active, given the group effects in both the acute and chronic testing, as outlined above.

There are several ways to address this dose issue for future studies in this area. The concentration of Flx may need to be adjusted for each individual rat. The methodology used by Silva and Brandao (2000) could be employed, in which drug solutions were prepared daily based on that particular cages' fluid consumption the day before. The current study adjusted the concentrations for each sex, but not for each individual bottle. Given the individual supply of fluid to each rat this would be an improvement on the current study. The possibility that Flx was slightly aversive to the rats (Prendergast et al., 1996) indicates that a vehicle other than drinking water may increase fluid consumption. For example, the water supply could be altered to a slightly sweetened fluid such as apple juice (LaRoche & Morgan, 2007). The use of injections was considered for the current study, but it was decided that the stress imposed on the animals to be injected for 28 days would be too great. However, injections would have allowed for more accurate doses. Thompson et al. (2004) suggested that osmotic minipumps could have been used instead of drinking water in their study. However, they reported that these minpumps would only be useful for the administration of the drug for two weeks. In addition, the extremely high cost of these minipumps was outside the budget of the current study.

The male and female rats within the current study were treated at the same time. There was an assumption of this treatment, that being, the periods in which the sexes experienced "adolescence" overlapped. However, it is well documented that females of non-rodent species reach puberty before

males, and some argue that the onset of puberty and associated chemical changes within the body may trigger the brain alterations characteristic of adolescence (Andersen, 2003). However, it was felt that during this early stage of adolescent-rat research, it was safer to utilize the same treatment period for both males and females, as the literature comparing male and female onset of adolescence/puberty in rats is scarce.

The current study employed the SIT, light-dark box and the Y-maze to investigate the effects of chronic Flx use on emotionality and short-term memory. An obvious query would we why one of the most common tests of emotionality, the EPM, was not used in the current study. The reasons are as follows. To date, there have been few studies utilizing the SIT and no studies using the light-dark box to investigate the effects of Flx. In addition, the EPM has been used extensively in the literature investigating the effects of a single dose or the acute effects of Flx, with very mixed results (for example, Silva et al., 1999; Griebel et al., 1999; Durand et al., 1999).

Another potential limitation of the current study is the lack of acute effects data for the E group. Although the E group was tested on the acute open-field, they were tested on PND 55 when all the other groups were tested but when the E subjects had been off Flx for 14 days. As such, any significant effects observed for the E group would have represented either the effects of a serotonin withdrawal syndrome or long term effects. One way to address this would have been to test all the groups on PND 41, on the last day of the E group's Flx treatment. However, the main focus of this thesis was on the long-term effects and so it was felt that this was not necessary.

As with any study utilizing animal research, the generalization of the results to human behaviour may be questioned. The brain and behaviour of rats do not reach the complexity evident in humans, and so the extent to which the results of this study can be generalized to humans is limited to the

extent that the brain and behaviour of humans and rats overlap. In addition, the rats in this study were PVG/C Hooded rats, which had not been developed as a model of human anxiety or depression. As such, there are limitations as to how much the results of the current study extend to the human population in general, and a human clinical sample more specifically.

6.6 Methodological Strengths

In addition to the methodological limitations outlined above, the current investigation contained a number of methodological strengths. A definite methodological strength of this study was the inclusion of the two testing ages, which allowed for the mapping of behavioural changes across time. Another key strength of the current study was the inclusion of both male and female subjects, as the majority of investigations into the effects of Flx have used only male subjects (Palanza, 2001).

The current study also contained a number of procedural strengths. The innovative inclusion of mesh separations within the home-cage of all rats allowed for the calculation of Flx dose for each individual rat, in the absence of isolated housing. This is important given the sex-specific effects found following isolated housing (Palanza, 2001). The procedures to which the rats were exposed to were consistent across groups, with all rats being handled and weighed at equal intervals across the treatment phase. In addition, despite the exclusion of the results of the E group from the acute open-field test, this group of rats was tested anyway for consistency in test exposure. That is, to ensure further tests and testing procedures were similar in their degree of familiarity across groups.

The use of rats as subjects for this investigation is a definite strength, as there would have been a number of limitations if human subjects had been used. First, the results of such an experiment employing humans as subjects would take many years. But the use of rats and their rapid life cycle enabled the current experiment to be conducted over several months. Secondly, if human subjects

were used then the natural disease process would no doubt confound the effects of Flx exposure. In addition, there is a possibility with a human clinical population that the individuals may have attempted to self-medicate in the past through the use of other psychoactive drugs, or they may have been polydrug users during the experiment. Such confounding variables were absent from a study such as this employing rat subjects. Thirdly, with high rates of suicide in a depressive sample, the use of human studies would likely be subject to high attrition rates and of course there would have been serious ethical issues conducting a randomized experiment such as this using human subjects.

7.0 General discussion

The findings of the current investigation supported the general finding that rats treated chronically with Flx display heightened anxiety-like behaviour as adults, relative to controls. In addition, treatment during the late phase of adolescence (PND 42-55), either alone or in combination with treatment during the early phase of adolescence (PND 28-42), appeared to lead to the most drastic behavioural consequences in adulthood.

7.1 Neurodevelopment

The findings of the current study support the growing body of literature documenting the long-term behavioural and neurophysiological effects following psychoactive substance use during adolescence (Smith, 2003; Andersen, 2005; Andersen & Navalta, 2004). These findings also support the idea that the adolescent brain is still developing, thus, is vulnerable to insult (Andersen, 2003). In particular, the increased 5-HT levels due to Flx administration appear to affect the brain in a way that leads to heightened emotional reactivity. Exactly how the brain was

affected by Flx cannot be determined from the results of the current study. However, it is highly likely that the serotonergic system was implicated.

Chronic Flx administration during adolescence has been associated with alterations in the hippocampus (Norrholm & Ouimet, 2000) and the frontal cortex (Wegerer et al., 1999) of rats. Norrholm and Ouimet (2000) documented an arrested dendritic spine proliferation within the CA1 region of the hippocampus, but not the dentate gyrus, following chronic exposure to Flx (5mg/kg/day) during the juvenile period (PND 21-41). It is noteworthy that this effect was observed both 24 hours and three weeks following the cessation of Flx exposure. The authors argue that the reduced dendritic spine density may be due to the effect Flx had on either the formation or retention of new dendritic spines. Wegerer et al. (1999) found that chronic oral administration of Flx (5mg/kg/day) via drinking water to male juvenile Wistar rats (PND 25-39) led to enduring region-specific alterations in the density of 5-HT transporters. Utilizing ligand-binding assays these researchers found an approximately 20% increase in the density of 5-HT transporters within the frontal cortex of rats treated during this juvenile period, a finding which was present at both PND 50 and later adulthood (PND 90) within the juvenile-treated group, but was absent within the control rats and rats treated at a later age (PND50-64).

7.2 Serotonin Hypothesis of Anxiety

The current study provides support for the 5-HT hypothesis of anxiety, which proposes that increased 5-HT levels are associated with anxiogenic effects, while reductions in 5-HT are associated with anxiolytic effects (Drapier et al., 2007). Immediately following chronic Flx treatment in adult rats, the levels of 5-HT and 5-H1AA have been reported to be below the levels of control subjects across a number of brain areas including the hypothalamus, amygdala,

prefrontal cortex, striatum and the hippocampus (Thompson et al., 2004; Durand et al., 1999). In accordance with this adult rat literature, it is plausible that during the acute open-field testing the adolescent subjects receiving Flx treatment experienced reduced 5-HT and 5-H1AA levels. As detailed in the above section, the rats that received Flx treatment during the acute open-field test displayed an anxiolytic behavioural profile, consistent with the 5-HT hypothesis of anxiety.

The long-term anxiogenic effects shown by the L and EL groups may also provide support for the 5-HT hypothesis of anxiety. In at least one study using adult male Sprague-Dawley rats it was found that, following a six day drug-free period, chronic Flx administration (PND 60-94) led to increased 5-HT levels (Brenes & Fornaguera, 2009). These authors found that within the hippocampus, the Flx group had the highest levels of 5-HT. Although there were no significant differences between the control and Flx groups for 5-HT, there was a trend toward the Flx treated group having the highest 5-HIAA levels. The results of the study by Brenes and Fornaguera (2009) in combination with the anxiogenic behavioural profile of the late phase Flx-treated rats in the current study, may be taken as evidence supporting the 5-HT hypothesis of anxiety.

7.3 Neuronal Imprinting Theory

The results of the current study support the neuronal imprinting theory. This theory postulates that behavioural and/or neurophysiological changes as a result of psychoactive substance exposure may endure (Andersen & Navalta, 2004). Additionally, these enduring effects may incubate, in that they are not observed immediately following drug exposure, but become apparent later on in life. In this study the acute Flx effects were anxiogenic. Conversely, the long-term effects of Flx were anxiolytic. If the serotonin hypothesis is correct, this suggests an

overall increase in 5-HT within the central nervous system in the long-term. However, the exact mechanism through which neuronal imprinting has occurred in the present study is not known. It is likely that the changed 5-HT levels had led to significant alterations in the serotonergic system within the brain, particularly those anatomical areas associated with anxiety, such as those structures which comprise the limbic system.

The current study also supports the idea of a 'sensitive period' as described by Andersen (2003). A sensitive period is a developmental phase in which specific factors, such as drugs, toxins and stress, influence the later development of that individual in a specific way. The sensitive period for the later anxiogenic effects due to Flx exposure in the PVG/C Hooded rat seems to fall during the late phase of adolescence, as defined within the current study as being between PND 42 and PND 55. This is based on the current findings that Flx exposure during the early phase of adolescence (PND 28-41) had no significant effects on anxiety-related behaviour, memory or social interaction. However, the rats treated during the late phase of adolescence displayed anxiogenic profiles during testing as adults (PND 70 and PND 120). More specifically, the sensitive period may fall at the later end of this phase, at approximately PND 50-55, given that LaRoche and Morgan (2007) found no significant anxiogenic effects following Flx exposure from PND 25-49.

7.4 Implications for Adolescence

The current findings regarding the long term effects of adolescent Flx use apply to rats, and caution should be used when attempting to generalise these results to a human population (Caccia, Cappi, Fracasso & Garattini, 1990). The complexity of the human adolescent phase cannot be replicated in an animal model. However, parallels in both neuroanatomical and

behavioural factors can be drawn. In addition, animal models can serve as a starting point for further research. If the current findings could be generalized to human adolescents, they would suggest that humans taking Flx during their later adolescent years may be at heightened risk for later anxiety issues. Of coarse, generalization of the current findings to humans is limited not only by the use of an animal model, but also the fact that humans taking Flx are generally doing so because of some underlying psychopathology, which has not been modeled in the current study.

8.0 Future Directions

The current study demonstrated a link between adolescent Flx use and later heightened emotionality. The current investigations were limited to tests of emotionality, social interaction and short-term memory, but there are many other areas of behavioural testing that would be of interest. In particular, the effects of adolescent Flx use on later risk for depression would be of interest, possibly utilising the forced swim test as a model. In addition, given the link between emotional disorders and substance abuse (Adamson, Todd, Sellman, Huriwai & Porter, 2006), it would be of interest to investigate whether this association could be due to the actions of serotonergic medications taken earlier in life. Future research into the neurophysiological mechanisms underlying the behavioural effects, or the mode of neuronal imprinting, would also be informative.

The main question left unanswered by the current study is whether or not these results can be extrapolated to human subjects. This would require a longitudinal investigation, comparing the trajectories of adolescents treated both with and without Flx. As outlined above, a study such as

this would contain several confounding variables such as disease effects, as well as the possible effects of other therapeutic interventions.

The current study should serve as a starting point for further research into the area of adolescent antidepressant use, and also contribute to the research attempting to define the adolescent period in rats. Being one of the first studies to compare early versus late phases of adolescence, the current study could serve as a model for further studies attempting to investigate sensitive periods. The methodology employed in the current study, specifically the use of drinking water to administer drugs, adds to the literature showing that this less invasive method can be used successfully (Wegerer et al., 1999; Silva & Brandao, 2000; Thompson et al., 2004). The current study also contributes to the literature documenting the effective doses of Flx required in rats to display behavioural and/or pharmacological effects, with the present investigation suggesting that doses within the range of 6-8 mg/kg/day are effective in PVG/C Hooded rats.

9.0 Conclusion

The current study supports the growing body of literature documenting the long-term behavioural and neurophysiological effects following psychoactive substance use during adolescence (Smith, 2003; Andersen, 2005; Andersen & Navalta, 2004). Although used as a treatment for anxiety disorders during adolescence, the current investigation suggests that adolescents taking Flx are at risk for later heightened anxiety. Interestingly, this heightened risk seems to be associated with Flx use only during the late phase of adolescence, suggesting that important neurodevelopmental changes are occurring within the serotonergic system during this phase.

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