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Effects of chronic paroxetine and fluoxetine treatment on markers of suicidal behavior in adolescent rats

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EFFECTS OF CHRONIC PAROXETINE AND FLUOXETINE TREATMENT ON MARKERS OF SUICIDAL BEHAVIOR IN ADOLESCENT RATS

A Thesis

Presented to the

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ABSTRACT

Major depression is a common problem in adolescents. Unfortunately, one the most effective antidepressants in adults, the selective serotonin reuptake inhibitor (SSRI) paroxetine, is not clinically effective in pediatric populations. Moreover, this compound can also increase rates of suicidal ideation and behavior. In contrast, the SSRI fluoxetine is an effective treatment for depression in adolescents and has a much lower risk for inducing suicidal ideation. The mechanism for the paradoxical increase in suicidal ideation after paroxetine treatment is unknown and limited research has been done on this topic. Thus, the goal of the present investigation was to compare the effects of paroxetine and fluoxetine in adolescent rats on markers associated with depression and suicidal behavior in humans. To this end, adolescent male and female rats were pre-treated with vehicle, paroxetine (2.5 or 10 mg/kg, IP), or fluoxetine (10 mg/kg, IP) for ten consecutive days starting on PD 35. The effects of fluoxetine and paroxetine on reactivity to rewarding stimuli and anxiety were assessed using sucrose preference and the elevated plus maze task, respectively. A separate group of adolescent rats received the same SSRI or vehicle pretreatment and BDNF protein and monoamine content was

assessed in various brain regions 24 h later. We found that 10 consecutive days of SSRI administration was sufficient to cause changes in neuronal function. Specifically, paroxetine treatment caused a significant reduction in serotonin concentrations in both the prefrontal cortex and hippocampus, while fluoxetine reduced serotonin levels in the prefrontal cortex. Paroxetine also caused a reduction in BDNF levels in males, while fluoxetine increased BDNF levels in females. Repeated treatment with these SSRIs caused only minor changes in sucrose consumption and had no effect on elevated plus maze behavior. These results suggest that SSRIs can cause significant changes in neuronal mechanisms associated with depression and suicidal-like behavior in adolescents. Interestingly, these changes in neuronal functioning did not translate into behavioral changes. While not conclusive, these data suggest that chronic treatment with the SSRI paroxetine may potentiate depression by reducing serotonin levels and decreasing BDNF levels.

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CHAPTER ONE

DEPRESSION IN ADOLESCENCE

Major depressive disorder (MDD) is a pervasive mental disorder that affects both adult and pediatric populations. This disorder is characterized by negative mood accompanied by sleep or appetite disturbances, delusions, irritability, anhedonia, impaired cognitive functioning and feelings of worthlessness or excessive guilt (Masi, Liboni, & Brovedani, 2010). There is also considerable evidence that MDD is positively correlated with comorbid psychopathology, substance abuse, and attempted suicide (Kovacs, 1996; Kovacs, Goldston, & Gastonis, 1993; Marttunen, Aro, Henriksson, & Lonnqvist, 1991; Rao, Weissman, Martin, & Hammond, 1993; Shaffer, 1988). This disorder is one of the most commonly diagnosed mental health disorders in the United States, with approximately one in every six individuals succumbing to clinical depression during their lifetime (Kessler, Chiu, Demler, Merikangas, & Walters, 2005). MDD is also evident in pediatric populations with a prevalence estimate of 2.5% (Birmaher et al., 1996). During adolescence the prevalence of MDD ranges between 4% and 8% and, astonishingly, the prevalence increases to 25% towards the

end of the adolescent period (Kessler, Avenevoli, & Ries Merikangas, 2001).

This high rate of MDD in adolescence is associated with an increased risk of suicidal behavior. A national survey on youth risk behaviors in high school students reported that 8.5% of this population self-reported that they had attempted suicide at some point in the past year. Interestingly, the majority of these suicide attempts did not use lethal methods (Grunbaum et al., 2004). Furthermore, 16.9% of this national sample reported seriously considering the act of suicide (Grunbaum et al., 2004). This self-report rate of suicide attempts and suicidal ideation is consistent with a 2006 report stating that suicide was the third leading cause of death for young people between the ages of 15 and 24 in the United States (Keller et al., 2001).

Pharmacotherapeutic Treatment of Major Depressive Disorder in Adolescent Populations

Due to the high rate of depression in pediatric populations, the use of antidepressants is increasing. In 2002, 11 million antidepressant prescriptions were written for adolescents and children within the United States (Rigoni, 2004). Unfortunately, clinical evidence for the efficacy and safety of antidepressant medications is

limited in adolescent populations and the existing data is often conflicting. Meta-analyses of antidepressant trials have indicated that tricyclic (TCA) antidepressants do not have a significant beneficial effect on depressive symptoms in pediatric populations (Ambrosini, Bianchi, Rabinovich, & Elia, 1993; Dujovne, Barnard, & Rapoff, 1995; Geller, Reising, Leonard, Riddle, & Walsh, 1999; Michael & Crowley, 2002; Varley, 2003; Wittington, Kendall, Fonagy, Cottrell, Cotgrove, & Boddington, 2004). In contrast, published clinical trials have indicated that serotonin reuptake inhibitors (SSRI) may be beneficial for the treatment of MDD in juveniles, but other reports have questioned the efficacy of these medications in pediatric populations (Emslie et al., 2002; Emslie, Rush, Weinberg, Gullion, Rintelmann, & Hughes, 1997; Keller et al., 2001; Simeon, Dinicola, Ferguson, & Copping, 1990; TADS, 2004; Wagner et al., 2003; Wagner, Robb, Findling, Jin, Gutierrez, & Heydorn, 2004). For instance, clinical trials have shown fluoxetine to be efficacious for children and adolescents with major depression when compared to placebo-treated groups (Emslie et al., 2004; Hetrick, Merry, McKenzie, Sindahl, & Proctor, 2007) Paroxetine treatment also significantly improves major depression in adolescents (Emslie & Mayes, 2001; Keller et al., 2001).

However, Emslie et al. (2006) has also reported that paroxetine-treated groups were not statistically different from placebo-treated groups.

Serotonin Reuptake Inhibitors and the Risk of Suicide in Pediatric Populations

Despite the evidence that SSRIs may be useful therapeutics in the treatment of MDD for juveniles, there are major concerns about their safety. Specifically, there is evidence that SSRIs may increase the risk of suicide in adolescents. In 2004, the FDA issued a "black box" label warning for all SSRI antidepressant medications used in children and adolescents. The warning addresses the potential increased risk of suicidal thought, tendencies, and behaviors in juveniles during short-term SSRI treatment for major depression. This warning also concluded that minors should be stringently monitored (for at least the first 4 weeks of treatment) for the emergence of predispositional symptoms to suicide, which include aggression, irritability, insomnia, and social withdrawal (FDA, 2004). The decision of the FDA was based on the results of a randomized controlled clinical trial, which indicated that there was a pronounced increase in suicidal behaviors and ideations in children not previously characterized with suicidal tendencies. Specifically,

suicidal behavior and ideations of children increased from 2% to 4% when treated with SSRIs (FDA, 2004; Hammad, 2004). The FDA later convened another meeting of its scientific advisory committee and determined that the black-box warning should be extended to late adolescence and early adulthood as well (Laughren & Levin, 2006). While the evidence that lead to this warning was provocative, it did not suggest that an individual's response to a particular antidepressant could be predicted with any certainty. It is still very much in question whether SSRI treatment increases the risk for suicide in adolescents. Unfortunately, the controlled clinical trials needed to adequately assess the increased risk of suicide would require a large number of participants to show even a small effect. It may not be plausible or ethical to control for a majority of the factors that may significantly impact the risk of suicide.

Thesis Statement

There are many variables that increase the risk of suicide including genetic, social, psychological, and biological factors, making the evaluation of the effect of SSRIs on suicidal behavior difficult to assess in human adolescents (Henry & Demotes-Mainard, 2006). Consequently,

an animal model that is not confounded by comorbid psychiatric disorders, biological or environmental influences would enhance our understanding of the function SSRIs in suicidal behavior during adolescence. Suicide is a complex behavior, which is impossible to reproduce in a single animal model. We can, however, duplicate aspects of suicide by investigating the characteristics associated with suicidal behavior, such as impulsivity, hopelessness, aggression, and irritability (Malkesman et al., 2009). To date, there has been very little research on the potential mechanisms whereby SSRIs increase the risk of suicide (Malkesman et al., 2009). Therefore, the purpose of this investigation was to assess the effects of SSRI (e.g., fluoxetine and paroxetine) administration on risk-taking and depressive behavior in adolescent rats. In addition, neurochemical levels that are critically altered in human suicide completers (e.g., serotonin and brain-derived growth factor) were also measured after chronic SSRI treatment.

CHAPTER TWO

SEROTONERGIC, ADRENERGIC, AND HYPOTHALAMIC-PITUITARY-ADRENAL AXIS DEVELOPMENT IN RATS

When is Adolescence?

Adolescence is a developmental stage characterized by greater levels of impulsivity, risk-taking, novelty-seeking, with reductions in anxiety and decreased stress reactivity compared to adults (Adriani, Seta, Dessi-Fulgheri, Farabollini, & Laviola, 2003; Spear, 2000). It also marks the transition from childhood to sexual maturity (Spear, 2000). The biological processes involved in reaching adolescence appear to be very similar across mammalian species including humans and rodents. The time scale for this development varies across species, because humans develop on a scale of months to years while rats develop on a scale of days to weeks. Humans spend an extended period of time in a prepubescent state and do not reach puberty until 11 years on to 16 years of age. Rats are born at a developmental stage which is roughly equivalent to the third trimester of a human fetus, with rats reaching sexual maturity (i.e., puberty) at approximately five weeks (McCormick & Mathews, 2007). Although the age range for adolescence in rats is not

universally agreed upon (Spear, 2000), the period between postnatal day (PD) 28 through 42 is a conservative range based on physiological and behavioral development (McCormick & Mathews, 2007; Spear, 2000). Other investigators suggest a broader range of adolescence that can start as early as PD 20 in females and extend until approximately PD 55 in males (Odell, 1990; Ojeda, Urbanski, & Ahmed, 1986).

Adolescence is marked by biological and behavioral changes which may have a pronounced impact on the efficacy of antidepressants. Therefore, this chapter will be dedicated to understanding the ontogeny of various systems involved in antidepressant action. One general pattern that emerges is that adrenergic systems develop much slower than serotonergic systems. This may explain why antidepressants function in a different manner in immature mammals when compared to adults (Murrin, Sanders, & Byland, 2007).

Development of the Serotonergic System

Serotonin or 5-hydroxytryptamine (5-HT) is an indolealkylamine synthesized within serotonergic neurons and is found throughout the nervous system. The cell bodies of serotonin neurons are mainly localized in the

mid- and hind-brain (Tork, 1990) and innervate almost the entire central nervous system (Rho & Storey, 2001). The serotonergic system plays a role in modulating a wide array of behavioral responses including aggression, mood, appetite and arousal (Kaye, 2008; Miller & O'Callaghan, 2006; Siever, 2008; Young & Leyton, 2002). In particular, it is thought that the rostral serotonergic projections are involved in the pathology of various psychiatric disorders (Jacobs & Azmitzia, 1992).

Considering the many roles of serotonin within the CNS, it is not surprising that this neurotransmitter system is one of the first to develop (Lauder, 1990; Rajaofetra, Sandillon, Geffard, & Privat, 1989; Rho & Storey, 2001). In neuroembryonic studies in rats, cells containing serotonin-positive neurons are observed as early as embryonic day 12, with serotonergic fibers detected at embryonic day 14 (Murrin et al., 2007; Wallace & Lauder, 1983). There is a rapid surge of neuronal and dendritic serotonergic growth after birth, which lasts until the end of the first postnatal week (Lidov & Molliver, 1982; Loizou & Salt, 1970), with adult serotonergic innervations patterns established by the third postnatal week (Dori, Dinopoulos, Blue, & Parnavelas, 1996; Lidov & Molliver, 1982). In the raphé

nucleus, synaptogensis of the serotonergic system is at 75% of adult levels by PD 15. The motor cortex continues to mature with neuronal density increasing until around PD 35 (Dori et al., 1996). The neuronal density of serotonin in the basal forebrain on the other hand, reaches adult levels at around postnatal week two but declines during the third week before increasing again (Dinopoulos, Dori, & Parnavelas, 1997). Ultimately, serotonin levels are low at birth and generally peak around PD 21 to 30 before decline to adult levels (Loizou, 1972; Whitaker-Azmitia, 2005).

Serotonin binds to six pharmacologically different G-coupled protein receptors, $5\text{-}HT_{-1,-2,-4,-5,-6,-7}$, and one ligand-gated ion channel receptor, $5\text{-}HT_3$ (Murrin et al., 2007). Several subtypes exist with each of the serotonin receptor families. The density of serotonin receptors and reuptake sites within the mammalian brain typically peak prior to puberty (i.e., pre-adolescent period [PD 21-30]), before decreasing to adult levels (Whitaker-Azmitia, 2005). For instance, serotonin receptor levels in the striatum are at only 12% of adult levels at birth, but rapidly increase to 150% by PD 21. The hippocampus and cortex, on the other hand, increase to only 75% of adult levels by PD 21 (Nelson, Herbet, Adrien, Bockaert, &

Hamon, 1980). By PD 15, 5-HT_1 receptor density in the brainstem is similar to adulthood (Whitaker-Azmitia, Lauder, Shemmer, & Azmitia, 1987). In contrast, 5-HT_2 receptors rapidly increase until PD 13 and then decline to adult levels (Roth, Hamblin, & Ciaranello, 1991). In the hippocampus, 5-HT_{5A} and 5-HT_7 receptors fluctuated during the pre-weanling period before stabilizing in adulthood (Garcia-Alcocer, Segura, Garcia, Martinez-Torres, & Miledi, 2006).

Development of the Adrenergic System

Norepinephrine is a member of the catecholamine family and functions as both a neurotransmitter and a hormone. Norepinephrine is synthesized from dopamine by the enzyme dopamine β-hydroxylase. In the peripheral nervous system, norepinephrine is produced by the adrenal medulla and is released into the circulatory system. In the central nervous system, noradrenergic neurons are localized in the locus coeruleus and lateral tegmental field (Rho & Storey, 2001). These neurons project to a wide array of brain regions, with the highest concentration of fibers extending to the hypothalamus (Rho & Storey, 2001). Adrenergic neurons from the locus coeruleus and lateral tegmental field also innervate the

spinal cord and cerebellum through a dorsal fiber bundle and nearly all of the norepinephrine is release into the cortex, limbic system, and thalamus originates from these regions.

Noradrenergic neurons begin to differentiate between embryonic day 10 to 13 in rats (Lauder & Bloom, 1974). From this point on, the adrenergic system follows a linear pattern of development within the central nervous system, proliferating 100- to 1000-fold by adulthood (Coyle, 1977). By PD 15, synaptogensis of norephinephrine neurons in the locus coeruleus is only at about half of adult levels (Lauder & Bloom, 1974). Noradrenergic projections and cell bodies appear during gestation but further maturation of cortical projections and synaptic formations continue to proliferate primarily during the first 3 weeks of age (Loizou, 1972). Generally, noradrenergic levels continue until around PD 30 to PD 40 when they reach adult concentrations. However, in the cerebellum and brainstem norepinephrine reaches maturation up to a week earlier than the rest of the system (Konkol, Bendeich & Breese, 1978).

Norephinephrine binds to three pharmacologically and molecularly different G-coupled protein receptors, α_1, α_2, and β, each with three subtypes, α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B},

α_{2c}, and β_1, β_2, β_3 (Rho & Storey, 2001). The α_1 receptors rapidly proliferate until PD 20, when the level of this adrenergic receptor decrease until reached adult levels. This overshoot correction may take weeks to occur. Beta receptors in the cerebral cortex develop very similarly, surpassing adult levels by the third postnatal week, and then slowly decline over the next few months (Murrin et al., 2007).

In conclusion, the serotonergic system reaches maturity at a much younger age, than the adrenergic system, an effect evident across mammalian species (Murrin et al., 2007). For example, the development of 5-HT innervation is completed during the pre-adolescent period in rats, which is two weeks earlier than the noradrenergic system. At birth, the uptake transporters for serotonin are fully developed, unlike NE which reaches adult levels 21 days later. The different maturation rates of norepinephrine and serotonin neurotransmitter systems suggest the possibility that psychoactive drugs may induce unique effects depending on age (Murrin et al., 2007). Consistent with this idea, clinical trials have repeatedly shown that TCA antidepressants are ineffective on children with major depressive disorder (Geller et al., 1999; Michael & Crowley, 2002; Varley, 2003; Wittington et al.,

2004). The underdeveloped noradrenergic system could definitely play a role in this finding (Murrin et al., 2007).

The Development of the Hypothalamic-Pituitary-Adrenal Axis

Adolescence is a period of maturation that prepares an animal for the transition into adulthood, a phase defined by the ability to sexually reproduce and survive independently (McCormick & Mathews, 2007). The HPA axis is important due to its involvement in the physiological reactions to environmental and psychological stressors. This system provides the ability to cope during aversive or threatening situations. Resent evidence has demonstrated a sensitivity period during adolescence where the HPA axis can influence neurological programming, such as increasing neuronal plasticity. This development period has only been shown previously in prenatal development (Grant, Compas, Stuhmacher, Thurm, McMahon, & Halpert, 2003; McCormick & Mathews, 2007; Spear, 2000).

The HPA axis modulates neuroendocrine system functioning through the actions of glucocorticoid hormones. Under the control of the hypothalamus, hormones are secreted from the paraventricular nucleus, including most notably arginine vasopressin (AVP) and corticotropin

releasing hormone (CRH) (Antoni, 1986). These hormones, in turn, control the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary, which then stimulates the secretion of glucocorticoids from the adrenal cortex (Keller-Wood & Dallman, 1984). Glucocorticoids play an important role in neuronal development and plasticity and can influence neurogenesis, synaptogenesis, neuronal excitability, and atrophy (McEwen, 2000). Glucocorticoids are also important in protein synthesis and metabolism of neurotransmitters (Keller-Wood & Dallman, 1984).

The purpose of the HPA axis is to regulate homeostatic mechanisms that after altered by stress-inducing events, thus preventing a potential overreaction to stressors which may have a deleterious impact on the organism. The HPA axis can be activated by a continuum of psychological and physiological stressors, both innate and learned (McCormick & Mathews, 2007). Glucocorticoids can facilitate attention, memory formation, activate energy stores, and improve cardiovascular tone to enhance an organism's ability to cope with a particular stressor. Conversely, prolonged release of these glucorticoids can cause robust effects on the peripheral and central nervous systems or an organism (McEwen, 2000). The HPA axis also has the ability to

self-regulate to protect against the harmful effects of prolonged exposure to glucorticoids. Glucocorticoids can inhibit the release of ACTH which in turn suppresses the release of more glucocorticoids and in the way, diminishes the physiological responses to a stress-inducing stimulus (Dallman, Akana, Jacobson, Levin, Cascio, & Shinsako, 1987; Keller-Wood & Dallman, 1984).

Components of the HPA axis reach adult levels at various points in development. Glucocorticoid receptors reaches maturity at around PD 14, much sooner than ACTH concentrations (Walker, Sapolsky, Meaney, Vale, & Rivier, 1986) and CRH-responsive cells (Senovilla, Garcia-Sancho, & Villalobos, 2005), which reach adult levels at about 60 days of age. Corticosterone levels reach adulthood at four weeks of age (McCormick & Mathews, 2007; Sapolsky & Meaney, 1986; Schoenfeld, Leathen, & Rabii, 1980). Consequently, the negative feedback system has not fully developed by preadolescence. The implication of this maturational lag is that stressful events will cause a delayed onset and prolonged release of corticosterone (Goldman, Winget, Hollingshead, & Levine, 1973). Investigators have also attributed a prolonged release of glucocorticoids to the undeveloped parvocelluar PVN (Viau, Bingham, Davis, Lee, & Wong, 2005). By two months of age,

ACTH- (Walker et al., 1986) and CRH-responsive cells (Senovilla et al., 2005) within the pituitary reach maturity.

CHAPTER THREE

NEUROBIOLOGY OF SUICIDAL BEHAVIOR

Several lines of evidence suggest that depression and suicide may be mediated by common mechanisms. For example, psychological autopsies in suicide completers have identified MDD as a major contributor to suicidal behavior (Van Heeringen & Marusic, 2003). In addition, the rate of suicide attempts is increased in people diagnosed with MDD when compared to the general population (Marttunen et al., 1991; Rao et al., 1993; Shaffer, 1988). However, other evidence suggests that suicide is a unique psychopathology and not simply a symptom of depression. In particular, the majority of people diagnosed with MDD will not attempt suicide, and some individuals who commit suicide do not suffer from MDD. Thus, it is clear that depression alone is not sufficient to explain suicidal behavior. Recently, other risk factors for suicidal behavior, such as impulsivity and aggression, have been identified. It is now clear that the neurobiological mechanisms of suicidal behavior probably involve a number of different distinct brain circuits.

Neurobiology of Depression

Brain Regions

Several brain regions and neuronal circuits that regulate emotion, reward, and executive functioning have been implicated in depression (Berton & Nestler, 2006). Brain regions such as the hippocampus and neocortex are believed to mediate the cognitive aspects of depression, which includes feelings of worthlessness, hopelessness, guilt, and suicidal tendencies (Nestler, Barrot, DiLeone, Eisch, Gold, & Monteggia, 2002). The nucleus accumbens, also called the ventral striatum, and the amygdala, are thought to be important for emotional memory (Nestler et al., 2002). FMRI studies on depressed patients have demonstrated abnormalities in the frontal cortex, cingulate cortex, hippocampus, amygdala, and striatum (Liotti & Mayberg, 2001; Manji, Drevets, & Charney, 2001; Zhu et al., 1999). Multiple meta-analyses have indicated that patients with recurrent depression have smaller hippocampal volumes (Campbell & MacQueen, 2006; Campbell, Marriott, Nahmias, & MacQueen, 2004; Videbech & Ravnkilde, 2004). This reduction is usually associated with memory deficits which are common among patients with mood disorders. Studies on amygdala volume provide inconsistent results; however, enlarged amygdala volume has been

reported much less frequently in patients with MDD (Bremner, Narayan, Anderson, Staib, Miller, & Charney, 2000; Frodl et al., 2002; MacMillan et al., 2003), than patients with smaller amygdala volume (Caetano et al., 2004; Hastings, Parsey, Oquendo, Arango, & Mann, 2004; Rosso, Cintron, Steingard, Renshaw, Young & Yurgelun-Todd, 2005; Sheline, Gado, & Price, 1998; Siegle, Konecky, Thase, & Carter, 2003). There is evidence that the striatum is smaller in some depressed adults (Greenwald et al., 1997; Husain et al., 1991; Krishnan et al., 1992).

Monoamine Hypothesis

Over 50 years ago, antidepressants revolutionize the clinical research field by indicating that depression is a disorder that could be treated by increasing synaptic neurotransmitter levels by inhibiting reuptake or preventing the degradation of released monoamines (Nestler et al., 2002). This finding plus studies showing that resperine, a drug that depletes monoamine stores, can induce depressive symptoms lead to the monoamine hypothesis. This hypothesis simply states that depression is caused by decreased monoamine activity.

Neurotrophic Hypothesis

While abnormalities in monoamine functioning are still considered to be important for depression, a growing

amount of research suggests that monoamine dysfunction cannot be the sole cause of depressive symptoms (Nestler et al., 2002). Evidence in support of this view include studies showing that: (1) monoamine depletion does not cause depression in most people (Ruhe, Mason, & Schene, 2007), (2) psychostimulants, like amphetamine and cocaine, increase levels of monoamines but do not alleviate symptoms of depression (Paul, 2001), and (3) acute administration of antidepressants, which induce an immediate increase in synaptic monoamine levels do not alleviate depressive symptoms (Berton & Nestler, 2006). An alternative to the monoamine hypothesis theory has recently been proposed that focuses on the dysfunctions in the neurotrophic factor, brain derived neurotrophic factor (BDNF). Neurotrophic factors are important for the development and maintenance of neurons and synaptic connections (Shirayama, Chen, Nakagawa, Russell, & Duman, 2002). The neurotrophic hypothesis of depression states that a deficiency in BDNF in specific brain regions, such as the hippocampus, results in depressive symptoms. This hypothesis is supported by animal research demonstrating that virtually all antidepressants up-regulate the expression of BDNF in the dentate gyrus and hippocampus (Nibuya, Morinobu, & Duman, 1995). This up-regulation of

BDNF has also been demonstrated in the hippocampus of humans (Chen, Dowlatshahi, MacQueen, Wang, & Young, 2001). In addition, animal research has demonstrated that infusions of BDNF into the midbrain, dorsal raphé nucleus, dentate gyrus, or hippocampus causes antidepressant-like responses in the forced swim test and learned helplessness model (Shirayama et al., 2002; Siuciak, Lewis, Wiegand, & Lindsay, 1997). Interestingly, the antidepressant effects of BDNF infusion actually persist longer than classical antidepressants (Shirayama et al., 2002).

Hyperactivity of the Hypothalamic-Pituitary-Adrenal Axis

Hyperactivity of the HPA axis is another alternative theory to the original monoamine hypothesis. The HPA axis is the primary system for reacting to acute and chronic stress. The HPA axis theory postulates that increased release of cortisol (also called hypercortisolism) produced by chronic stress leads to depressive symptoms. Under stress, cortisol and other glucocorticoids cause the hippocampus and paraventricular nucleus to exert a powerful inhibition feedback on the HPA axis to regulate and promote healthy cognition (Nestler et al., 2002). However, prolonged cortisol release can damage hippocampal neurons resulting in reduction in dendritic arborization

(Nestler et al., 2002). Moreover, some studies have shown that depressed patients have reduced hippocampal volumes compared to non-depressed controls (Bremner et al., 2000; Sheline, Sanghavi, Mintun, & Gado, 1999). Hyperactivity of the HPA axis in depression is supported by evidence that a large majority of individuals exhibit aberrant, over activation of the HPA axis, which can be reduced through antidepressant administration (Arborelius, Owens, Plotsky, & Nemeroff, 1999; De Kloet, De Kock, Schild, & Veldhuis, 1988; Holsboer, 2001; Sachar & Baron, 1979).

Adolescent Depression

Adolescent depression is associated with a constellation of endocrine, physiological, chemical and morphometric neurological abnormalities (Davidson et al., 2002). The symptoms of major depression are expressed differently depending on maturational stage but, in general, poor social, cognitive, and interpersonal skills, as well as a significant risk for self-harm, are characteristics common among all ages (Reed, Happe, Petty, & Byland, 2008). Adolescent FMRI studies have revealed that males with MDD have a smaller volume and reduced white matter in the left anterior cingulate cortex compared to control (Hastings et al., 2004). Post-mortem studies also indicate that the anterior cingulate cortex

of depressed adolescents is reduced in size (Campbell & MacQueen, 2006). Adolescents with depression have smaller bilateral hippocampal volumes when compared to healthy controls (Hickie et al., 2005).

Neurobiology of Impulsivity and Risk-Taking

Kochansky (1973) demonstrated that after presenting a depressive mood-stimulating film, suicide attempters exhibited a greater preference for risk-taking compared to suicide threateners, non-suicidal psychiatric patients or healthy controls. Clinical investigations have revealed several brain regions involved in risk-taking decision processes. Lesions of the prefrontal cortex and orbitofrontal cortex both lead to impaired decision making abilities on a risk-taking task (Bechara, Damasio, Tranel, & Damasio, 1997; Rogers et al., 1999). FMRI studies on healthy adults during a risk-taking task have shown activation in the orbitofrontal cortex, inferior prefrontal cortex, ventrolateral and ventromedial cortecies, insula, and parietal regions (Critchley, Mathias, & Dolan, 2001; Elliott, Dolan, & Frith, 2000; Elliott, Rees & Dolan, 1999; Liu, Powell, Wang, Gold, Corbly & Joseph, 2007; Paulus, Rogalsky, Summons, Feinstein, & Stein, 2003). Activation of the anterior

cingulate cortex and lateral prefrontal cortex have been implicated in the regulation of inhibitory control (i.e. impulse control) when making a risk-taking decision (Lee, Chan, Han, Leung, Fox, & Gao, 2008).

Adolescent Risk-Taking Behavior

During adolescence, dramatic neurobiological changes occur to brain regions associated with risk-taking. These changes begin with a massive growth of synaptic and axonal fiber densities in the amygdala and prefrontal cortex (Cunningham, Bhattacharyya, & Benes, 2002). These fibers continue to grow and expand until the late-adolescent period (Cunningham et al., 2002). During this late-adolescent period, dendritic pruning rapidly increases in the amygdala, nucleus accumbens, and prefrontal cortex (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Teicher, Andersen, & Hostetter, 1995; Zehr, Todd, Schulz, McCarthy, & Sisk, 2006). This rapid synaptic pruning during late-adolescence is believed to occur in response to the overproduction that took place during the start of adolescence. Furthermore, there is a time-dependent difference between the development of the limbic sub-cortical and frontal cortical regions. In particular, the prefrontal cortex is the last region to undergo active myelination (Andersen et

al., 2000; Giedd, 2004; Teicher et al., 1995). Overall, regions specific to emotion and executive functioning are still developing throughout the adolescent period. Furthermore, the late development of these important regions may underlie the heightened emotional reactivity and sensitivity due to the without the ability to override inappropriate thoughts and behaviors mimicking the under developed sub-cortical (Casey, Jones, & Hare, 2008).

Central Nervous System Abnormalities Associated with Suicidal Behavior

Hypofunctionality of the serotonergic system is a common finding in suicide completers (Mann, 2003). For example, postmortem studies have found lower levels of both serotonin and 5HIAA (the main metabolite for serotonin) in brain stem tissues, along with lower level of 5HIAA in the cerebrospinal fluid (Mann et al., 1996). Investigators have also found abnormalities in the serotonergic system with decreased presynaptic serotonin transporter sites in the occipital cortex, hypothalamus, brainstem, and prefrontal cortex (Mann, Arango, Marzuk, Theccanat, & Reis, 1989; Mann, Henteleff, Lagattuta, Perper, Li, & Arango 1996). The ventral-medial prefrontal cortex, an area related to impulsivity and aggression, has also been shown to have less serotonergic transporter

binding, in all cortical layers (Arango, Underwood, Gubbi, & Mann, 1995; Mann, 2003).

Evidence of noradrenergic system impairment in suicide completers has also been reported. Specifically, the number of noradrenergic neurons are reduced in the locus *coeruleus* and lower brainstem of suicide completers (Arango, Underwood, & Mann, 1996; Ordway, Widdowson, Smith, & Halaris, 1994). Moreover, compensatory increases in prefrontal α_2, and β-adrenergic receptors, and tyrosine hydroxylase activity have been reported (Mann, 2003). This overactivity of the noradrenergic system and HPA axis is associated with severe anxiety and agitation (Fawcett, Busch, Jacobs, Kravitz, & Fogg, 1997).

Postmortem studies have shown that levels of BDNF may be region specific with significant reductions in the prefrontal cortex and hippocampus of suicide completers (Karege, Vaudan, Schwald, Perroud, & La Harpe, 2005). In addition, the BDNF receptor, TrkB, is also significantly reduced in the prefrontal cortex and hippocampus of suicide completers compared to normal controls (Karege et al., 2005).

CHAPTER FOUR

SEROTONIN REUPTAKE INHIBITORS AND ADOLESCENCE

The rate of SSRIs prescribed for adolescents has risen over the last decade, despite the risk of suicidal ideation and hostility induced by the administration of these drugs (Clavenna, Bonati, Rossi, De Rosa, 2004; FDA, 2004; Murray, de Vries, Wong, 2004; Schirm, Tobi, Zito, & de Jong-van den Berg, 2001). This increase in SSRI prescriptions is thought to be due in part to the poor efficacy and side effects of alternative treatments, such as tricyclic antidepressants (Hazell, O'Connell, Heathcote, & Henry, 2002). A recent meta-analysis of published and unpublished double-blind clinical trials, revealed an increased risk of suicidal thoughts, behaviors and adverse effects for children and adolescents prescribed SSRI medications compared to those who received placebos (Hetrick et al., 2007). Fluoxetine, however, is the only SSRI that was consistently effective in attenuating depression symptoms (Hetrick et al., 2007). Thus, fluoxetine is the only medication to have an acceptable risk-benefit ratio when compared to all other SSRIs (Cheung, Emslie, & Mayes, 2005). Interestingly, another widely used medication in the pediatric

population, paroxetine, has demonstrated no clinical efficacy in treating depression (Cheung et al., 2005). Hence, this chapter discusses the neurochemical and behavioral effects of fluoxetine and paroxetine within the adolescent population.

SSRIs are classified by their ability to selectively increase extracellular levels of serotonin by inhibiting presynaptic reuptake and are at least 10-fold more selective for serotonin than for norepinephrine reuptake (Feighner & Boyer, 1996). Fluoxetine was the first SSRI medication to be approved by the FDA for the treatment of depression and the first to be found efficacious for the treatment of major depression in children and adolescents (Emslie et al., 1997; 2004). The major side effects associated with tricyclic antidepressants, such as drowsiness, ataxia and memory loss are not exhibited by fluoxetine, because it has very weak binding affinity to histaminergic and cholinergic receptor sites (Hyttel & Larsen, 1985).

Paroxetine (trade names, Paxil and Seroxat) was the third SSRI to be approved by the FDA for the treatment of depression in adults and is one of the most widely prescribed antidepressant medications in the United States. A phenylpiperidine derivative, paroxetine is

chemically unrelated to any other antidepressants currently in use (Bourin, Chue, & Guillon, 2001). Paroxetine is one of the most specific and potent SSRIs with the highest affinity for serotonin transporters (Mellerup & Plenge, 1986; Owens, Morgan, Plott, & Nemeroff, 1997). By comparison, fluoxetine has only a tenth of the affinity that paroxetine possesses for serotonin transporters (Owens et al., 1997). Paroxetine also has a moderate affinity for norepinephrine transporters (Nemeroff & Owens, 2003). Like fluoxetine, paroxetine is considered a relatively "clean" drug in comparison to older tricyclic compounds and monoamine oxidase inhibitors because it has very little to no binding affinity in rats to the adrenergic (α_1, α_2), dopamine (D_1, D_2), serotonergic ($5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$), histamine (H_1) receptors and a weak affinity to the noradrenaline and muscarinic acethylcholine receptors (Hyttel, 1994; Owens et al., 1997).

Effects of Repeated Serotonin Reuptake Inhibitors Administration in Adolescents Rats

Although it is believed that fluoxetine is overall effective in adolescent psychiatry, there are reports of adverse drug effects such as suicidality, agitation, worsening of depression and anxiety within this population

(March *et al.*, 2004). Furthermore, there is a deficit in research looking at not only the effect of fluoxetine on behavioral and neurochemical alterations, but the effect that other widely used SSRIs, such as paroxetine, may have. This section will highlight the effects of fluoxetine and paroxetine in various areas of research.

Neurochemical Alterations

Rats administered fluoxetine (5 mg/kg, oral) for two weeks beginning on PD 25, but not PD 50, had an increase in serotonergic transporter densities in the frontal cortex when compared to rats treated with vehicle (Wegerer, Moll, Bagli, Rothenberger, Ruther, & Huether, 1999). The absence of an effect in rats administered the same dose during the end of the adolescent period (PD 50) indicates that fluoxetine treatment has a unique effect when given prior to late-adolescence. The density of noradrenergic transporters in the frontal cortex remained unchanged for both groups (Wegerer et al., 1999).

The "high-anxiety/stress" mouse strain, BALB/cJ, exhibited lower levels of serotonin and 5HIAA after a two week administration of fluoxetine during adolescence, relative to the "low-anxiety/stress" C57BL/6J mouse strain (Anisman, Haylay, Kelly, Borowski, & Merali, 2001; Norcross et al., 2008). There were also lower hippocampal

volumes in the BALB/cJ strain compared to C57BL/6J strain but no significant difference in frontal cortex size (Norcross et al., 2008).

Alterations in the neurogenesis of hippocampal neurons have been implicated in both the pathology and treatment of depression (Jacobs, Praag, & Gage, 2000). Norrholm and Ouimet (2000) found that three weeks of chronic fluoxetine during adolescence (PD 21-45) arrested age-associated dendritic spine development in the CA1 hippocampus. In contrast, acute treatment with fluoxetine increased dendritic length and spine density (Norrholm & Ouimet, 2000). These results were surprising because chronic fluoxetine treatment in adults has been shown to increase cell proliferation in the hippocampus (Huang & Herbert, 2006; Malberg, Eisch, Nestler, & Duman, 2000; Marcussen, Flagstad, Kristjansen, Johansen, & Englund, 2008).

In contrast to fluoxetine, there have been no chronic paroxetine studies in adolescent rodents that assess neurochemical changes. Thus, it is unclear at this point, if paroxetine alters markers of suicide or depression.

Behavioral Alterations

Very few behavioral changes have been found after adolescent exposure to chronic fluoxetine in rodents. No

significant differences were found in the duration spent on open arms using an elevated plus maze after fluoxetine treatment during postnatal week three through seven (LaRoche & Morgan, 2007). In addition, Norcross and colleagues (2008) found that administering clinically relevant doses of fluoxetine to adolescent mice (3-7 weeks) did not cause any significant changes on assays for fear-, anxiety- and stress-like behaviors when tested in adulthood under drug-free conditions. Furthermore, adolescent administration of fluoxetine did not produce any alterations in anxiety-related behaviors in adulthood, such as on the elevated plus-maze or forced swim task. Despite two early accounts that fluoxetine treatment reduced conditioned fear after adolescent treatment (Burghardt, Sullivan, McEwen, Gorman, & LeDoux, 2004), some researchers have dismissed these accounts as not being consistent with the general trend (Norcross et al., 2008).

Thirty-day chronic administration of paroxetine beginning on PD 33 caused a reduction in body weight that is hypothesized to be the result of reduced food consumption (de Jong, Pattij, Veening, Waldinger, Cools, & Olivier, 2005). This reduced consumption was believed to be caused by the activation of 5-$HT_{1B/2C}$ receptors

(Simansky, 1996). The same chronic paroxetine regimen also caused anxiety-like performance on the elevated plus maze when tested in adulthood.

Pharmacokinetics of Serotonin Reuptake Inhibitors in Adolescence

Fluoxetine is currently the only SSRI with an active metabolite, norfluoxetine, which exhibits clinical relevance. Norfluoxetine is not only a potent antidepressant; it also inhibits cytochrome P450 isoenzyme (Fuller, Snoddy, Krushinski, & Robertson, 1992). In humans, fluoxetine has a half-life of six days, with a 14 to 21 day half-life for its active metabolite (Newhouse, Ko, & Richter, 1996). Paroxetine has a significantly shorter half-life of 10 to 33 hours (Goodnick & Goldstein, 1998). Fluoxetine also exhibits a slower onset of therapeutic efficacy compared to paroxetine (De Wilder, Spiers, Mertens, Bartholome, Schotte & Leyman, 1993). The extended half-life of fluoxetine appears to be advantageous in protecting against sporadic noncompliance, a missed dose or the occurrence of withdrawal phenomena (Stokes & Holtz, 1997). Furthermore, Weiss and Gorman (2005) found trends across pharmacokinetics of different antidepressants. The results of their work demonstrated that those medications with shorter half-lives generally

having an increased risk of suicidal ideations and behaviors.

It is important to consider the clinical relevance of an antidepressant administered to rodents. To cause a significant level of serotonin transporter inhibition within the CNS, the minimum therapeutic dose of fluoxetine for humans is 1 mg/kg (Wegerer et al., 1999; Wong, Bymaster, Reid, & Threlkeld, 1983). Because rats metabolize fluoxetine roughly ten times faster than humans; the clinically equivalent dose for rat should range around 5 to 15 mg/kg (LaRoche & Morgan, 2007). Fluoxetine has been administered to rodents orally (Santarelli et al., 2003), by intraperitoneal injection (Kodama, Fujioka, & Duman, 2004; Malberg et al., 2000; Marcussen et al., 2008), and by osmotic pumps (Huang, Bannerman, & Flint, 2008; Huang & Herbert, 2006). Most commonly, fluoxetine has been administered intraperitoneally at 5 mg/kg (Cowen, Takase, Fornal, & Jacobs, 2008; Norrholm & Ouimet, 2000) and 10 mg/kg (Mason et al., 2009; Reed et al., 2008). Enhancement of hippocampal neurogenesis or proliferating cell survival was not altered by prolonged administration of fluoxetine (5 mg/kg) (Cowen et al., 2008). However, acute treatment of fluoxetine (5 mg/kg) did increased dendritic length and

spine density (Norrholm & Ouimet, 2000). Rats treated with fluoxetine (10 mg/kg) had a significant decrease in depressive-like behaviors using the forced swim task (Reed et al., 2008).

Paroxetine on the other hand, has been used in a range of different dosages, from 0.1 mg/kg up to 12 mg/kg in rodents (Casarotto & Andreatini, 2007; De Vry, Maurel, Schreiber, de Beun, & Jentzsch, 1999; Draiper et al., 2007; Gervasoni et al., 2002; Konkle, Sreter, Baker & Bielajew, 2003). However, there is very little research done in the adolescent rodents, therefore the range doses provided include adult studies as well.

CHAPTER FIVE

THESIS PROPOSAL AND HYPOTHESIS

Major depression is not an uncommon problem in human adolescents and those adolescents who suffer from clinical depression are at a much greater risk of committing suicide (Shaffer et al., 1996). Unfortunately, many of the medications that are effective in adults in relieving the symptoms of depression are ineffective in adolescent populations. The most popular class of antidepressants, the SSRIs, can induce suicidal thought in adolescents. The mechanism for this paradoxical increase in suicidal ideation and behavior is unknown and limited research has been conducted on this topic. Thus, the goal of the present investigation was to assess the chronic effects of SSRI treatment in adolescent rats on behavioral and neurochemical measures associated with depression and suicidal behavior in humans. Paroxetine and fluoxetine were the SSRIs investigated. Paroxetine was chosen because it is highly prone to increase suicidal behavior in humans (Tiihonen, Lonnqvist, Wahlbeck, Klaukka, Tanskanen, & Haukka, 2006). Moreover, paroxetine has shown very little clinical efficacy in reducing the symptoms of depression in adolescents (Emslie et al., 2006; Tiihonen et al.,

2006). Fluoxetine was chosen as a comparator, because it has a much lower risk value for inducing suicidal behavior and is an effective treatment for depression in human adolescents. Rats were treated with paroxetine (2.5 or 10 mg/kg), fluoxetine (10 mg/kg) or vehicle for ten consecutive days starting on PD 35. This injection period was chosen because it is analogous to middle adolescent period in humans (Spear, 2000). Separate groups of rats were used for the behavioral and neurochemical assays and testing began 24 hours after the last drug administration.

Behavioral measures were assessed on a sucrose preference task and an elevated plus maze tasks. The sucrose preference task, which measures reward sensitivity or anhedonia, is a choice procedure where rodents are given an opportunity to drink from a bottle containing water sweetened with sucrose or a bottle of tap water. This measure was chosen because it is sensitive to procedures that induce depressive symptoms in rats (i.e., unpredictable shock and neonatal isolation) and to antidepressant treatment (Katz, 1982). In addition, the sucrose preference task does not involve aversive stimuli, is easily measured, and can be tested repeatedly. The second measure was the elevated plus maze task. The elevated plus maze procedure is the most common method for

measuring anxiety in rodents and is also used as a measure of impulsivity (Dawson & Tricklebank, 1995; Handley & McBlane, 1993; Meyer, Piper, Vancollie, 2006; Rodgers & Cole, 1994). The elevated plus maze procedure involves placing rats on an elevated apparatus shaped like a plus, with two enclosed arms and two open arms. The amount of time spent in the open and closed arms is measured. The more time spent in the open arms indicates lower levels of anxiety and greater levels of impulsivity. The measure was chosen because it is a well-validated measure and can be completed in a short period of time. We hypothesized that repeated treatment with paroxetine would decrease sucrose preference and increase the amount of time spent in the open arm of the elevated plus maze when compared to vehicle- and fluoxetine-treated rats. We also hypothesized that repeated fluoxetine treatment would not alter either behavioral measure.

The neurochemical measures included monoamine content and BDNF levels within the prefrontal cortex and hippocampus. Monoamine levels were measured by high performance liquid chromatography with electroelectrochemical detection (HPLC-EC). BDNF levels were measured by enzyme-linked immunosorbent assays (ELISA). We expected that chronic paroxetine treatment

would decrease serotonin and BDNF levels when compared to vehicle- and fluoxetine-treated rats. We expected that chronic fluoxetine would not alter any of our neurochemical measures when compared to vehicle-treated rats.

CHAPTER SIX

METHODS

Animals

A total of 130 male and female rats (n = 5-9 per group) of Sprague-Dawley descent (Charles River Laboratories, Wilmington, MA), born and raised at California State University, San Bernardino, were used. Litters were culled to 10 pups on PD 3 and housed with the dam until PD 25. After weaning, rats were housed (4-6 rats per cage) with same-sex littermates. Rats in the behavioral experiment were single housed for the duration of the sucrose preference test. The colony room was maintained at 22-24° C and kept under a 12-hr light/dark cycle. All animals were treated according to the "Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council, 2003) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Apparatus

The elevated plus maze was made of black plastic and consists of two perpendicular, intersecting runways (110.5 × 10.2 cm) located 70 cm above the floor (San Diego Instruments, CA). One of the runways contains two "closed

arms" with 30.5 cm high walls enclosing the planks and the other runways have no walls or "open arms". The plus maze was placed in the center of a quiet dimly lit room (30 watt bulb) that was equipped with a video camera positioned above the maze.

Drugs

Fluoxetine hydrochloride and paroxetine hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO). Both fluoxetine and paroxetine were dissolved in dimethyl sulfoxide (DMSO) and injected intraperitoneally (IP) at a volume of 1 ml/kg.

In Vivo Drug

Rats received paroxetine (2.5 or 10 mg/kg), fluoxetine (10 mg/kg) or vehicle once daily from PD 35 to PD 44.

Experiment 1: Sucrose Preference and Elevated Plus Maze

Sucrose Preference Procedure

Four days before beginning drug injections rats were trained on the sucrose preference task using a two bottle choice test. On the first day, rats were singly housed and habituated to drinking from two bottles both containing water for one day. On the next three days, one of the water bottles was replaced with a bottle containing a 2%

sucrose solution. The bottles were weighed and refilled each day at the same time in the morning. The position of the bottles was switched each day to avoid position preferences. Following the initial sucrose procedure, rats were returned to group housing. After the last drug injection, rats were returned to individual housing and a final sucrose test given. Sucrose preference was defined using the following formula: [volume of sucrose ingested/ (the volume of water ingested + volume sucrose ingested) × 100] (Rygula, Abumaria, Flugge, Fuchs, Ruther, & Havemann-Reinecke, 2005; Strekalova, Spanagel, Bartsch, Henn, & Gass, 2004).

Elevated Plus Maze Procedure

Immediately following the sucrose test, rats were individually brought into the test room and placed in the center of the maze facing the open arm. Testing lasted 5 min. Time(s) in the open and closed arms, as well as entries, was scored from the videotapes. A similar elevated plus maze procedure has been described previously (Bolaños, Barrot, Berton, Wallace-Black, & Nestler, 2003; Frussa-Filho, Barbosa-Junior, Silva, Da Cunha, & Mello, 1999; Patisaul & Bateman, 2008). All four paws crossing the threshold defined entire into open or closed arms and

entire into the center of the maze was defined by only two paws crossing.

Experiment 2: Serotonin Content and Brain Derived Neurotrophic Factor Assays

Twenty-four hours after the last drug injection, rats were rapidly decapitated and their prefrontal cortex, hippocampus, and amygdala removed. The tissue sections for each animal were divided into two sections and frozen at -80°C until time of assay. Trunk blood samples were also collected for plasma extraction.

Serotonin Content Assay

Frozen tissue samples from the prefrontal cortex and hippocampus were sonicated in 5 volumes of 0.1N HClO4 and centrifuged at 20000 g for 30 min at 4°C. The supernatant was then filtered through a 0.22 mm centrifugation unit at 2000 g for 5 min at 4°C. Twenty microliters of the resulting extracts were then assayed for 5-HT using high performance liquid chromatography (582 pump and an MD-150 column; ESA, Chelmsford, MA) with electrochemical detection (Coulochem II; ESA). The mobile phase consisted of 75 mM NaH_2PO_4, 1.4 mM 1-octane sulfonic acid, 10 mM EDTA, and 10% acetonitrile [(pH 3.1) MD-TM Mobile Phase; ESA] and were pumped at a rate of 0.5 ml/min.

Brain Derived Neurotrophic Factor Enzyme-Linked Immunoassay

BDNF levels in the hippocampus and amygdala were assessed using the Promega BDNF E_{max} Immunoassay System (Promega, Madison, WI). Briefly, prefrontal, amygdala, and hippocampal tissue were homongenized in distilled water and sonicated for 15 s. Samples were then centrifuged at 16000 × *g* for 30 min and resulting supernantant collected. Standard 96-well flat-bottom Corning ELISA plates were incubated with carbonate coating buffer containing monoclonal anti-BDNF overnight at 4°C. The next day, the plates were blocked with 1× TBST buffer for 1 hr at room temperature. Serial dilutions of known amounts BDNF ranging from 500 to 0 pg was performed in duplicate for the standard curve for each rat sample. For both the standards and the samples, 100 µl was added to each well in duplicate and incubated 2 hr at room temperature. The wells were then be incubated with a secondary anti-human BDNF polyclonal antibody for 1 hr at room temperature. Then, the wells were incubated with anti-IgY conjugated to HRP for 1 hr at room temperature. A TMB solution was used to develop color in the wells for 10 min at room temperature. The reaction was stopped with the addition of 1 N HCl to the wells.

Statistical Design and Analysis

Two-way (drug × sex) analyses of variance (ANOVAs) were used to analyze the data from elevated plus maze and neurochemical (BDNF and monoamine content assay) experiments. Tukey or Dunnett post hoc tests ($p < 0.05$) were conducted as necessary. The independent variables for all experiments was drug treatment (fluoxetine 10 mg/kg, paroxetine 2.5 mg/kg, paroxetine 10 mg/kg, or vehicle)and sex (male or female) The dependent variable, for the elevated plus maze paradigm was number of entries and time spent on the open arms. Separate statistical analyzes were conducted for each of the three different brain regions (hippocampus, amygdala, and frontal cortex) used in the neurochemical assays.

Baseline sucrose preference was analyzed by a 4 × 2 × 3 (drug × sex × test day) repeated measures ANOVA. Sucrose preference after SSRI treatment was analyzed with a 4 × 2 (drug × sex) ANOVA. Side preference on the water habituation day was analyzed by a (sex × side) repeated measures ANOVA. Sucrose preference was defined using the following formula: [volume of sucrose ingested/ (the volume of water ingested + volume sucrose ingested) × 100].

In order to control for litter effects, no more than one subject from a litter was assigned to a particular group. An equal number of male and female rats were assigned to each group.

CHAPTER SEVEN

RESULTS

Effects of Selective Serotonin Reuptake Inhibitors on Body Weight

During the injection period (i.e., PD 35-44) male and female rats exhibited a progressive increase in body weight [day main effect, $F(9,1107) = 686.922$, $p < 0.001$] (see Figure 1). This increase in body weight was altered by SSRI treatment, sex and day [drug x day x sex interaction, $F(7,116) = 39.440$, $p < 0.001$](see Figure 1). Specifically, fluoxetine (10 mg/kg) significantly decreased weight gain on PD 39-40 and PD 42-44 in female rats and on PD 40-44 in male rats when compared to same sex saline-treated controls [Tukey test, $p < 0.05$]. In addition, paroxetine (10 mg/kg) significantly decreased weight gain on PD 41-44, but only in male adolescent rats. The lower dose of paroxetine did not significantly affect the body weights of either male or female rats.

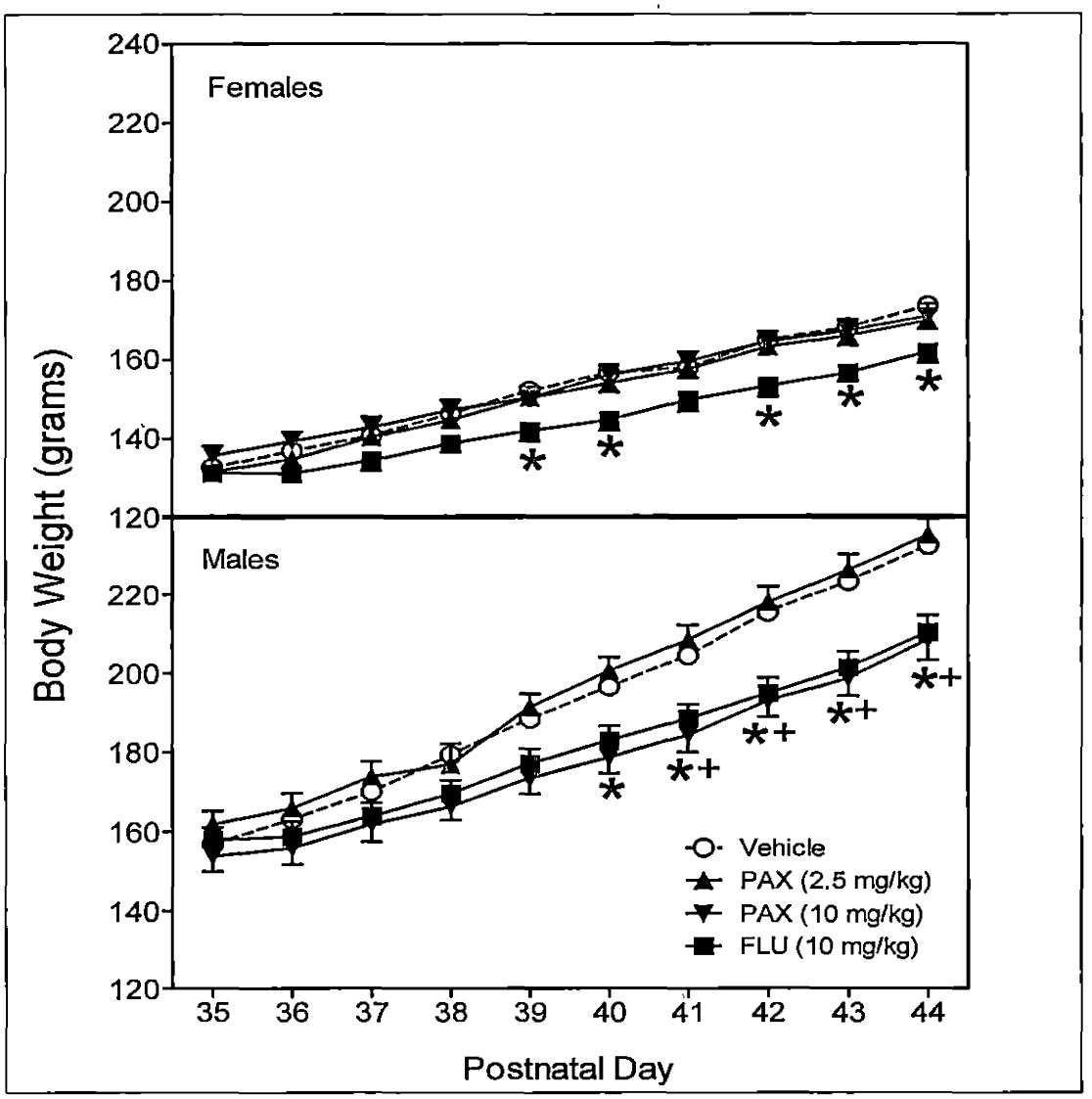

Figure 1. Mean Body Weight in Grams (±SEM) of Male (n = 14-19) and Female (n = 13-16) Adolescent Rats Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44. *Indicates a Significant Difference between Rats Treated with Fluoxetine (10 mg/kg) and Rats Treated with Vehicle. +Indicates a Significant Difference in Male Rats Treated with Paroxetine (10 mg/kg) Compared to Vehicle-Treated Male Rats

Effects of Serotonin Reuptake Inhibitors on Serotonin and 5HIAA Levels in the Prefrontal Cortex and Hippocampus

Adolescent SSRI treatment altered the levels of serotonin in the prefrontal cortex [drug main effect, $F(3,45) = 7.994$, $p < 0.001$] (see Figure 2). Chronic treatment with both paroxetine (10 mg/kg) and fluoxetine reduced serotonin levels when compared to vehicle-treated controls [Tukey test, $p < 0.05$]. Levels of serotonin were also reduced after paroxetine (10 mg/kg) treatment compared to rats treated with paroxetine (2.5 mg/kg) [Tukey test, $p < 0.05$]. Serotonin levels for rats treated with paroxetine (2.5 mg/kg), however, did not differ from saline-treated controls.

SSRI treatment also altered the levels of the primary serotonin metabolite, 5HIAA, in the prefrontal cortex [drug main effect $F(3, 44) = 3.790$, $p < 0.05$]. Fluoxetine reduced 5HIAA levels when compared to both vehicle-treated controls and rats given the lower dose of paroxetine (2.5 mg/kg) [Tukey test, $p < 0.05$]. Similar to 5HIAA levels, serotonin utilization or turnover (i.e., 5HIAA/5HT) was also altered by SSRI treatment [drug main effect $F(3, 44) = 3.803$, $p < .05$] (Figure 3). Rats treated with paroxetine (10 mg/kg) had greater serotonin turnover when compared to fluoxetine-treated rats. However, neither the

paroxetine- nor fluoxetine-treated groups differed significantly from vehicle-treated rats.

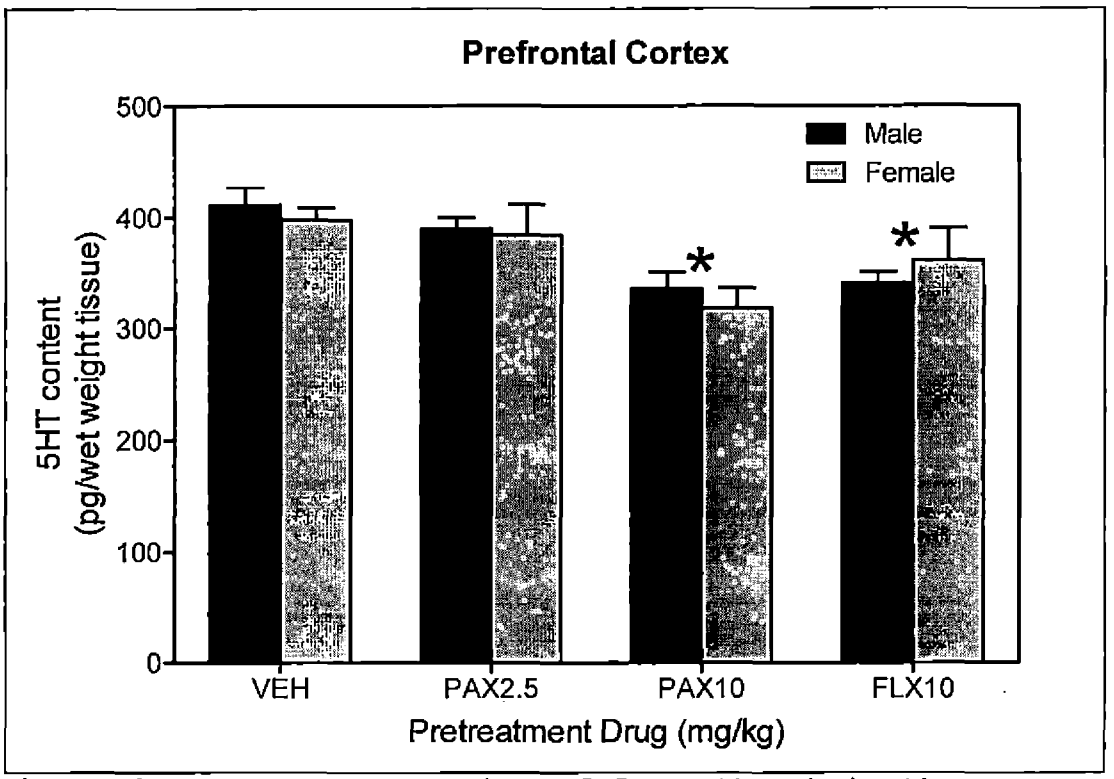

Figure 2. Mean Concentration of 5-HT (±SEM) in the Prefrontal Cortex of Adolescent Male (n = 8) and Female (n = 6-8) Rats 24 h after Last Drug Injection. Rats were Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44. Results are Expressed in Picograms per Milligram (pg/mg) of Wet Weight Tissue. *Indicates a Significant Reduction Compared to Vehicle-Treated Rats ($p < 0.05$)

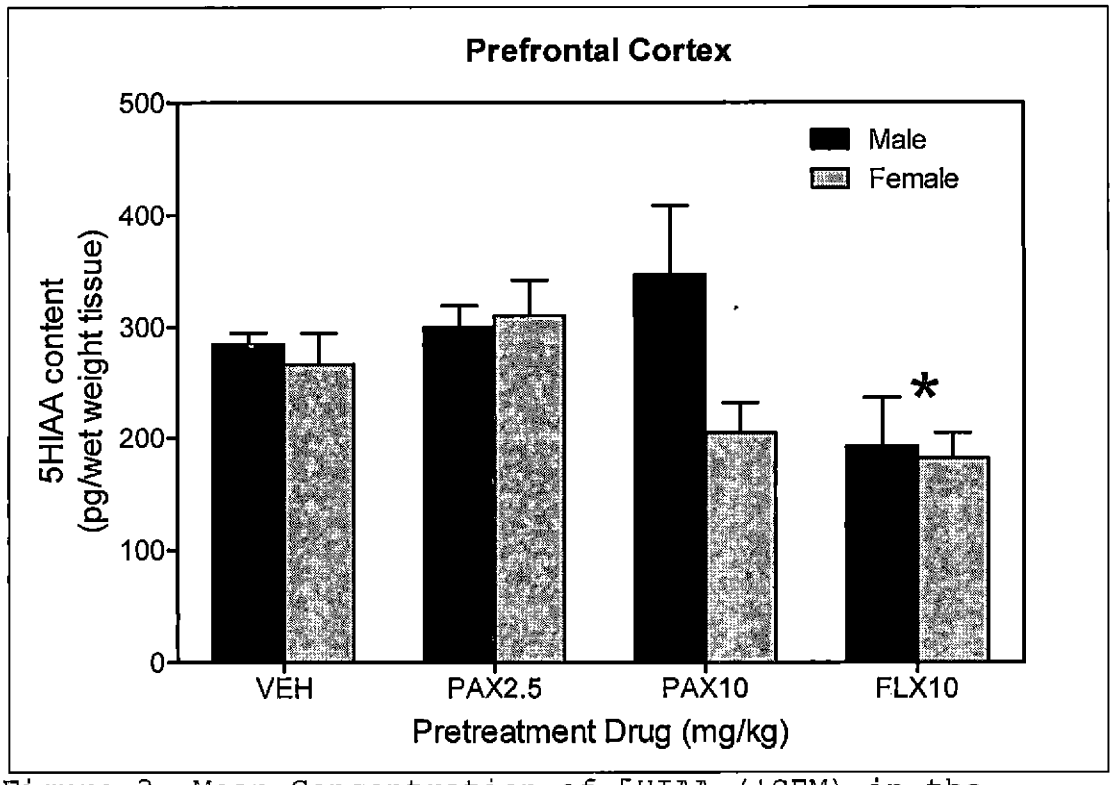

Figure 3. Mean Concentration of 5HIAA (±SEM) in the Prefrontal Cortex of Adolescent Male (n = 8) and Female (n = 6-8) Rats 24 h after Last Drug Injection. Rats were Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44. Results are Expressed in Picograms per Milligram (pg/mg) of Wet Weight Tissue. *Indicates a Significant Reduction Compared to Vehicle-Treated Rats ($p < 0.05$)

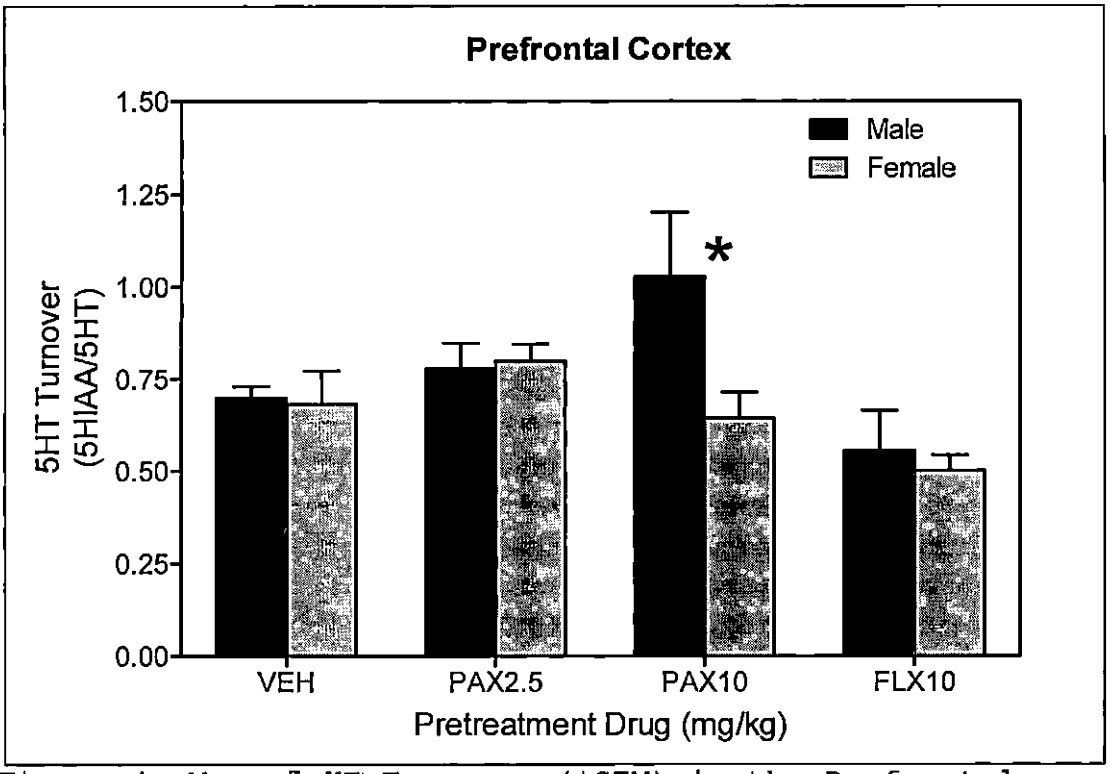

Figure 4. Mean 5-HT Turnover (±SEM) in the Prefrontal Cortex of Adolescent Male (n = 8) and Female (n = 6-8) Rats 24 h after Last Drug Injection. Rats were Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44. Results are Expressed in Picograms per Milligram (pg/mg) of Wet Weight Tissue. *Indicates a Significant Increase Compared to Vehicle-Treated Rats ($p < 0.05$)

Serotonin levels in the hippocampus were decreased by treatment with paroxetine (10 mg/kg) in both male and female rats [drug treatment main effect, $F(3,48) = 3.290$, $p < 0.03$, Tukey Test, $p < 0.05$] (see Figure 4).

Interestingly, the serotonin metabolite, 5HIAA, was reduced by both paroxetine and fluoxetine treatment in a sex-dependent manner [sex x drug treatment interaction, $F(3, 48) = 4.26$, $p < 0.01$]. Specifically, female rats showed decreased 5HIAA levels after both paroxetine (10 mg/kg) and fluoxetine treatment while male rats only showed a decrease after fluoxetine treatment [Tukey test, $p < 0.05$]. Turnover of serotonin (i.e., 5HIAA/5HT) was reduced after fluoxetine treatment [drug treatment main effect, $F(3, 48) = 6.403$, $p < 0.001$, Tukey test, $p < 0.05$] (Figure 5). The lower dose of paroxetine (2.5 mg/kg) did not affect serotonin activity.

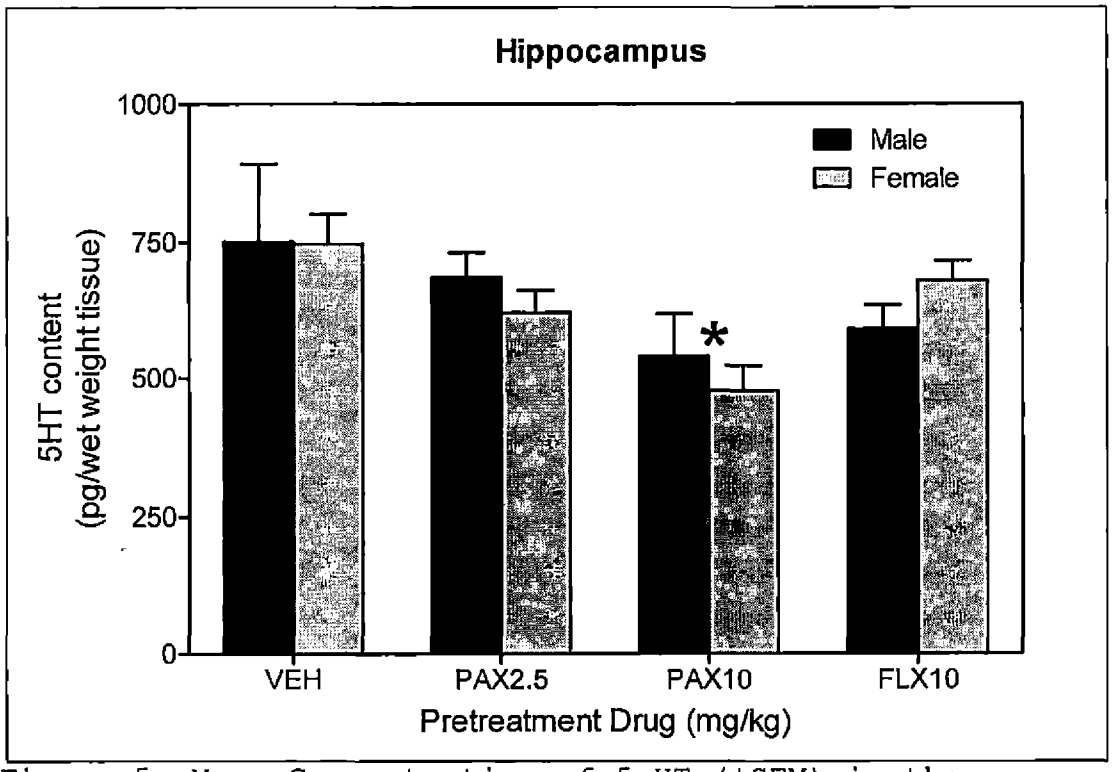

Figure 5. Mean Concentration of 5-HT (±SEM) in the Hippocampus of Adolescent Male (n = 8) and Female (n = 6-8) Rats 24 h after Last Drug Injection. Rats were Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44. Results are Expressed in Picograms per Milligram (pg/mg) of Wet Weight Tissue. *Indicates a Significant Reduction Compared to Vehicle-Treated Rats ($p < 0.05$)

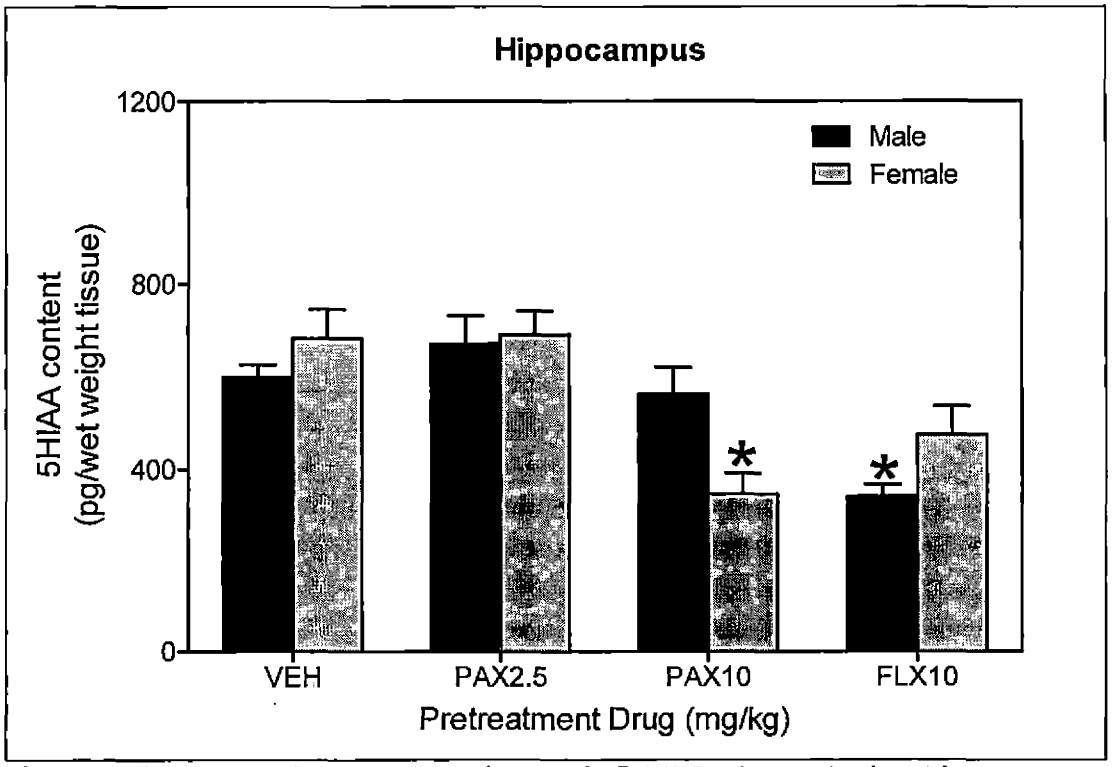

Figure 6. Mean Concentration of 5HIAA (±SEM) in the Hippocampus of Adolescent Male (n = 8) and Female (n = 6-8) Rats 24 h after Last Drug Injection. Rats were Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44. Results are Expressed in Picograms per Milligram (pg/mg) of Wet Weight Tissue. *Indicates a Significant Reduction Compared to Opposite Sex ($p < 0.05$)

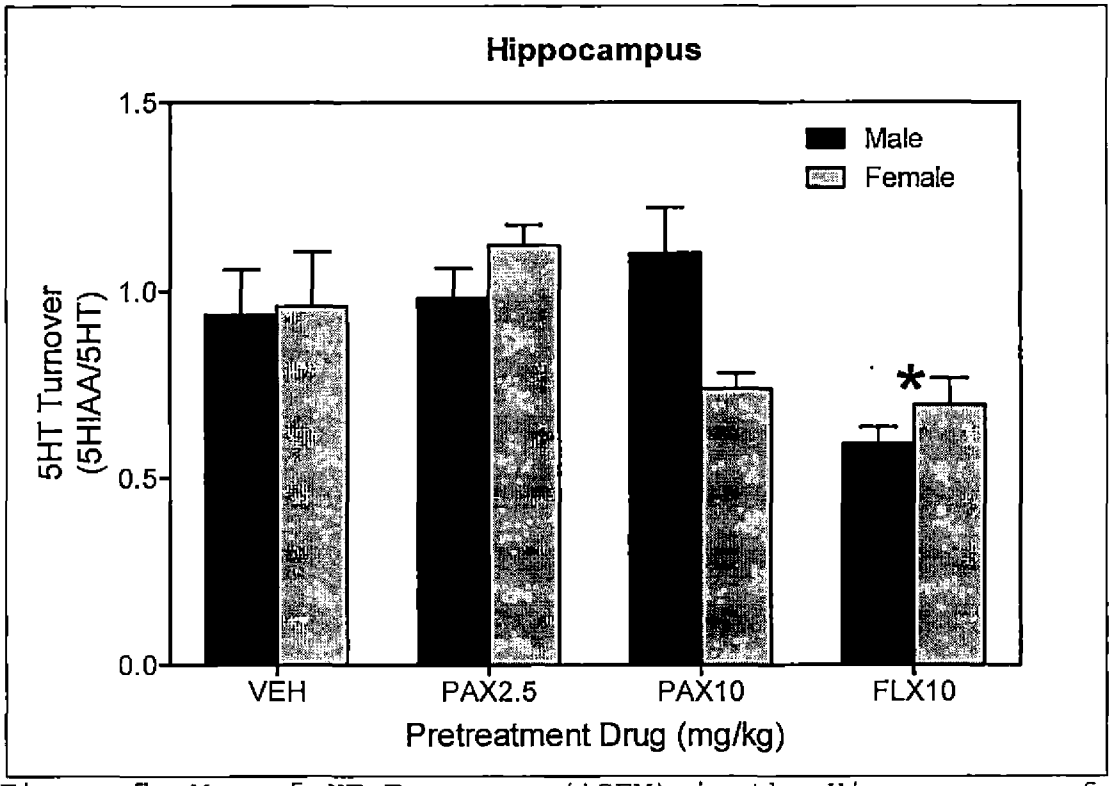

Figure 7. Mean 5-HT Turnover (±SEM) in the Hippocampus of Adolescent Male (n = 8) and Female (n = 6-8) Rats 24 h after Last Drug Injection. Rats were Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44. Results are Expressed in Picograms per Milligram (pg/mg) of Wet Weight Tissue. *Indicates a Significant Reduction Compared to Vehicle-Treated Rats ($p < 0.05$)

Effects of Serotonin Reuptake Inhibitors on Brain Derived Neurotrophic Factor Levels in the Hippocampus and Amgydala

Hippocampal BDNF levels were altered by SSRI treatment in a sex-dependent fashion [sex x drug treatment

interaction, $F(3, 39) = 3.782$, $p < 0.02$] (see Figure 6). In female rats, BDNF levels were increased by fluoxetine treatment, while male rats were unaffected [Dunnett test, $p < 0.05$]. In contrast, paroxetine treatment decreased BDNF levels in male rats, with no effect on the BDNF levels of female rats [Dunnett test, $p < 0.05$]. There was no significant difference in BDNF levels in the amygdala after repeated SSRI treatment.

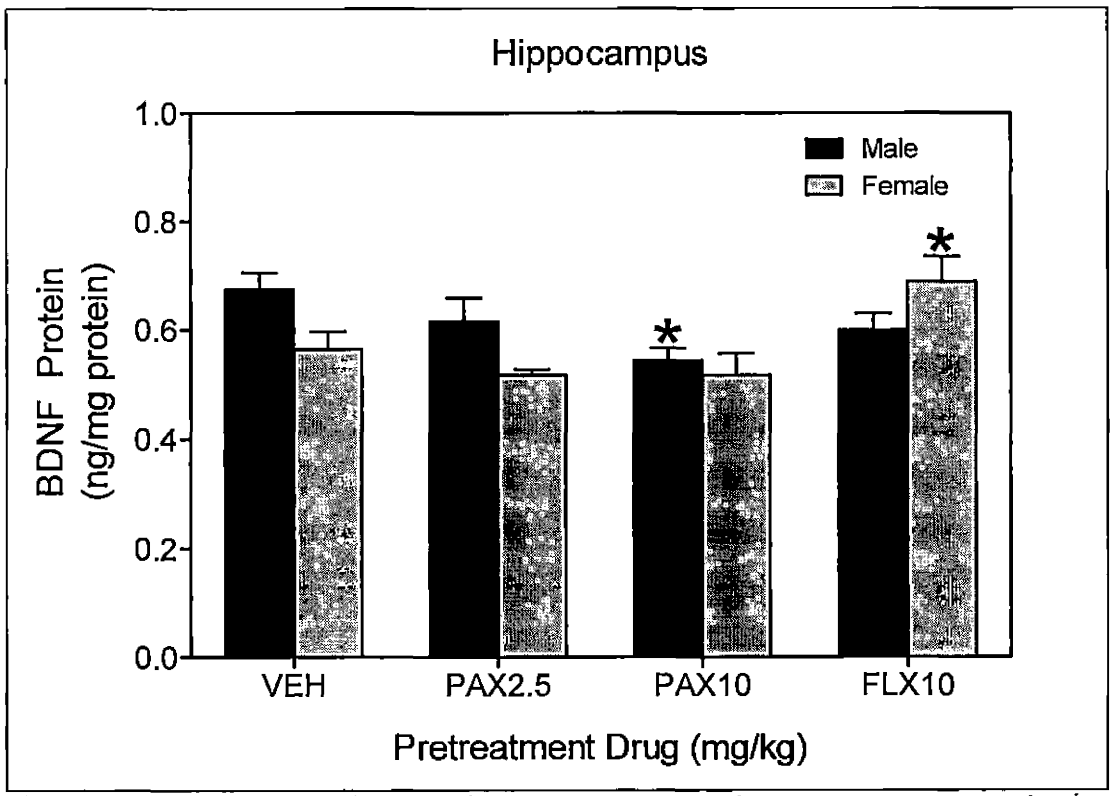

Figure 8. Mean Brain Derived Neurotropic Factor Protein Content (±SEM) in the Hippocampus of Adolescent Male (n = 6-7) and Female (n = 5-6) Rats 24 h after Last Drug Injection. Rats were Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44. Results are Expressed in Nanograms per Milligram (ng/mg) Protein. *Indicates a Significant Difference from Same Sex Vehicle-Treated Rats ($p < 0.05$)

Effects of Serotonin Reuptake Inhibitors on Sucrose Preference

On the water habituation day (i.e., first day of training), male rats (M = 23.836 g, SEM ± 0.40) drank more

water than female rats (M = 21.034 g, SEM ± 0.38) [sex main effect, $F(1, 62) = 26.152$, $p < 0.001$]. Moreover, both male and female rats drank more water from the right side (M = 12.768 g, SEM ± 0.58) than the left side (M = 9.579 g, SEM ± 0.51) on the water habituation day [side main effect, $F(1, 62) = 9.566$, $p < 0.005$. Before SSRI treatment there was no difference between male and female rats in the total amount of water or sucrose consumed. There was also no difference in preference scores over the three baseline preference days. Total sucrose consumption was greater for both male and female rats on Baseline day 1 (M = 35.515 g, SEM ± 2.44) than on Baseline Days 2 and 3 (M = 30.752 g, SEM ± 2.46; M = 31.831 g, SEM ± 2.54, respectively). After 10 days of SSRI treatment, there was no difference in total water or sucrose consumed when measured on the final test day. In addition, a Pre/Post preference score (Final preference score - Average of 3 baseline preference scores) showed that the drug treatment did not affect sucrose preference. However, the total amount of sucrose consumed was affected by SSRI treatment, because rats treated with the lower dose of paroxetine (2.5 mg/kg) consumed greater levels of sucrose when compared to rats that received the higher dose of paroxetine (10 mg/kg) or fluoxetine (10 mg/kg) [drug treatment main effect $F(3, 46) = 3.286$,

$p < 0.03$, Tukey Test, $p < 0.05$] (see Table 1). However, none of the drug treatment groups differed significantly from the vehicle-treated rats.

Table 1. Effects of Selective Serotonin Reuptake Inhibitor Treatment on Sucrose Consumption, Water Consumption and Sucrose Preference in Adolescent Male and Female Rats. Rats were Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44.

	Average Pre-Exposure		Post-Exposure		Pre/Post Preference Score
	Sucrose	Water	Sucrose	Water	
Male					
Vehicle	33.12 (±3.5)	1.87 (±0.4)	38.31 (±5.8)	6.19 (±2.7)	0.88 (±0.1)
Paroxetine (2.5 mg/kg)	36.64 (±5.0)	3.38 (±1.6)	48.41 (±10.7)	3.72 (±1.4)	1.03 (±0.1)
Paroxetine (10 mg/kg)	26.59 (±5.1)	5.13 (±3.4)	33.25 (±7.0)	6.38 (±3.6)	1.09 (±0.1)
Fluoxetine (10 mg/kg)	26.11 (±5.3)	6.81 (±2.7)	33.44 (±7.1)	10.08 (±4.1)	0.99 (±0.2)
Female					
Vehicle	33.13 (±8.6)	4.93 (±1.5)	50.44 (±13.0)	4.88 (±2.4)	1.00 (±0.1)
Paroxetine (2.5 mg/kg)	31.35 (±8.0)	6.87 (±2.6)	63.45* (±15.5)	3.09 (±0.80)	2.84 (±1.7)
Paroxetine (10 mg/kg)	32.13 (±5.2)	4.71 (±1.8)	36.45 (±9.4)	5.75 (±2.3)	0.93 (±0.0)
Fluoxetine	29.67	5.73	34.54	4.62	1.12

(10 mg/kg)	(±6.5)	(±2.7)	(±5.7)	(±2.7)	(±0.1)

*Indicates a significant difference from rats treated with 10 mg/kg paroxetine or 10 mg/kg fluoxetine ($p < 0.05$).

Effects of Serotonin Reuptake Inhibitors treatment on Elevated Plus Maze Behavior

There was no effect of sex or drug treatment on any of the measures assessed on the elevated plus maze during the 5 min testing period (see Table 2).

Table 2. Effects of Selective Serotonin Reuptake Inhibitor Treatment on Elevated Plus Maze Behavior of Adolescent Male and Female Rats. Rats were Treated with Vehicle, Paroxetine (2.5 or 10 mg/kg), or Fluoxetine (10 mg/kg) from PD 35 to 44.

	Duration in closed arms(s)	Duration in open arms(s)	Ratio	Closed arm entries	Open arm entries	Ratio
Male						
Vehicle	139.49 (±9.8)	57.98 (±10.0)	0.42	10.25 (±1.2)	5.13 (±0.9)	0.50
Paroxetine (2.5 mg/kg)	137.09 (±20.1)	66.02 (±15.0)	0.48	10.25 (±0.5)	6.75 (±1.6)	0.66
Paroxetine (10 mg/kg)	126.15 (±12.0)	91.55 (±7.8)	0.73	10.29 (±0.8)	6.86 (±1.7)	0.67
Fluoxetine (10 mg/kg)	137.26 (±9.0)	64.85 (±9.3)	0.47	10.38 (±1.0)	5.63 (±0.7)	0.54
Female						
Vehicle	131.50 (±11.4)	73.70 (±11.0)	0.56	10.57 (±1.2)	6.71 (±0.8)	0.64
Paroxetine (2.5 mg/kg)	141.71 (±13.3)	63.66 (±10.5)	0.45	10.56 (±1.3)	5.78 (±1.0)	0.55

Paroxetine (10 mg/kg)	124.07 (±12.2)	80.63 (±12.4)	0.65	10.38 (±1.5)	6.13 (±0.6)	0.59
Fluoxetine (10 mg/kg)	123.98 (±9.6)	79.91 (±11.3)	0.64	10.88 (±1.2)	6.88 (±1.1)	0.63

CHAPTER EIGHT

DISCUSSION

Introduction

Fluoxetine and paroxetine are commonly prescribed for the treatment of major depressive disorder in pediatric populations. In this population, paroxetine is not efficacious and has been shown to increase rates of suicidal behavior (Emslie et al., 2006; Tiihonen, Lonngvisy, Wahlbeck, Klaukka, Tanskanen, & Haukka, 2006). In contrast, fluoxetine is an effective treatment for depression in adolescence and adulthood and has a much lower risk for inducing suicidal behavior (Ipser, Stein, Hawkridge, & Hoppe, 2009; Lovrin, 2009; Masi, Liboni, & Borovedani, 2010). The reason for the paradoxical increase in suicidal behavior in pediatric populations is unknown but may be the result of age-dependent differences in the neuronal response to paroxetine. Thus, the goal of the present investigation was to compare the effects of paroxetine and fluoxetine in adolescent rats using neurochemical and behavioral markers associated with depression and suicide in humans. Our results revealed that 10 consecutive days of SSRI administration was sufficient to cause changes in neuronal activity.

Specifically, paroxetine treatment caused a significant reduction in serotonin concentrations in both the prefrontal cortex and hippocampus, while fluoxetine only reduced serotonin levels in the prefrontal cortex. Paroxetine also caused a reduction in BDNF levels in the hippocampus, while fluoxetine increased BDNF levels. These antidepressants, however, only caused minor alterations on depression- and anxiety-related behavioral tasks, because neither elevated plus maze behavior or sucrose preference was altered after SSRI treatment.

Effects of Selective Serotonin Reuptake Inhibitors on Body Weight

Consistent with both human and rodent studies, fluoxetine treatment led to significantly reduced body weight in both male and female adolescent rats (de Jong et al., 2006; Konkle & Bielajew, 1999, Mancini & Halpern, 2006; McNamara, Able, Rider, Tso, & Jandacek, 2010; Mitchell, De Zwaan, & Roerig, 2003; Ward, Comer, Haney, Fischman, & Foltin, 1999). This result may be explained by multiple factors including increased activation of 5-$HT_{1/2}$ receptors and the increased synaptic availability of serotonin causing appetite suppression (Halford, Harrold, Boyland, Lawton, & Blundell, 2007; Simansky, 1996).

Interestingly, chronic treatment with paroxetine (10 mg/kg) significantly reduced weight gain in adolescent males but not females. This indicates that there is a sex-specific difference between the two SSRIs in their effect on the serotonergic activity associated with weight gain. The ability of fluoxetine and paroxetine as anorectic agents is not correlated with their potency as serotonin reuptake inhibitors, because fluoxetine is less potent than paroxetine in blocking serotonin reuptake. This indicates that the anorectic effect of SSRIs may be mediated by other mechanisms. Lastly, the lower dose of paroxetine (2.5 mg/kg) did not affect weight gain in either sex, consistent with other studies (Konkle & Bielajew, 1999).

Neurochemical Markers of Depression and Suicide

The present results confirm and extend previous research demonstrating that chronic fluoxetine administration can significantly decrease serotonin and 5HIAA levels in adolescent prefrontal cortex (Caccia, Fracasso, Garattini, Guiso, & Sarati, 1992; Fuller, Snoddy, & Robertson, 1988). Serotonin concentrations in the prefrontal cortex were reduced after paroxetine (10 mg/kg) treatment to levels that are similar to

fluoxetine-treated rats. Moreover, fluoxetine treatment caused an overall reduction in cortical 5HIAA, whereas paroxetine (2.5 or 10 mg/kg) was without significant effect. This suggests that while serotonin levels are reduced after paroxetine administration, the metabolism of serotonin remains unaffected. Levels of hippocampal serotonin were reduced only by the higher dose of paroxetine (10 mg/kg). Serotonin levels were not affected by chronic fluoxetine treatment in the hippocampus; however, serotonin turnover was significantly reduced. Interestingly, hippocampal 5HIAA levels were altered by the SSRIs in a sex-dependent manner. 5HIAA levels were reduced in females treated with paroxetine (10 mg/kg) or fluoxetine, but only males rats treated with fluoxetine exhibited reductions in hippocampal 5HIAA. The mechanism by which administration of SSRIs reduce serotonin content is unknown. It is possible, however, that this decrease in serotonin content was due to either (a) increased sensitivity of the serotonin autoreceptors or (b) down stream feedback mechanisms.

Repeated SSRI treatment caused a significant sex-dependent effect on BDNF concentrations in the hippocampus. Fluoxetine (10 mg/kg) treatment in female rats significantly increased the concentration of BDNF,

while paroxetine (10 mg/kg) significantly decreased BDNF concentration in males. The ability of fluoxetine to increase BDNF in adolescent females mimics the results found in adult females given a similar dose (Hodes, Yang, Van Kooy, Santollo, & Shors, 2010). Moreover, the finding that fluoxetine did not alter BDNF levels in adolescent males, is in agreement with studies done in adult males (Alter, Whitehead, Chen, Wortwein & Madsen, 2003; De Foubert et al., 2004). Currently, the only available data on BDNF activity after SSRI treatment in adolescence is indirect, because cell proliferation was measured and not BDNF levels. Cell proliferation is an expression of neurogenesis, which is mediated by BDNF protein. In adolescent rats, fluoxetine exposure has no effect on cell proliferation (Cowen et al., 2008; Hodes, Yang, Van Kooy, Santollo, & Shor, 2009).

To our knowledge, the effects of paroxetine on BDNF levels in adolescents have not been previously investigated. In the present study, we found a reduction in BDNF after paroxetine exposure in males. Adult studies have demonstrated an increase in BDNF gene expression after repeated treatment of paroxetine (Coppell, Pei, & Zetterstrom, 2003). However, Coppell et al. (2003) used a longer administration regimen, which may have resulted in

a heightened BDNF response. Increases in BDNF concentrations may not directly elevate mood, but rather enhance BDNF signaling in mood-regulating circuits (Castren, 2005; Groves, 2007). Further research is necessary to elucidate the mechanism by which repeated SSRI treatment affects neurogenesis.

Behavioral Markers of Depression and Suicide

Overall, paroxetine and fluoxetine did not have a robust effect on behavioral measures associated with depression- and suicide-related behaviors. For example, sucrose consumption and preference, measures of hedonia in animals, were not affected by SSRI treatment. However, sucrose consumption was significantly increased in female rats after chronic administration of paroxetine (2.5 mg/kg) when compared to paroxetine (10 mg/kg) and fluoxetine (10 mg/kg) treatment in female rats. In males, chronic fluoxetine treatment during adolescence can cause slight increases in sucrose preference at very low sucrose concentrations (Iniguez, Warren, & Bolanos-Guzman, 2010). This finding implies that administering SSRIs during adolescence may minimally alter sensitivity to natural reward.

It is important to note that both paroxetine and fluoxetine treatment can induce nausea (Limebeer, Litt, & Parker, 2009; Tuerke, Leri, & Parker, 2009). However, the doses used in our study have not been shown to induce a significant increase in aversive taste reactivity. Moreover, SSRIs have been shown to reduce caloric intake and the palatability of sweet solutions (Asin, Davis, & Bednarz, 1992; Halford & Blundell, 2000; Halford, Harrold, Lawton, & Blundell, 2005; Simansky & Eberle-Wang, 1993). However, our study did not show an effect of SSRI administration on consumption of sucrose. Overall, it does not appear that sucrose intake was affected by a reduction in weight or an aversive gustatory response.

Neither chronic administration of paroxetine or fluoxetine changed the behavior of adolescent rats on the elevated plus maze. There was a trend for male rats treated with paroxetine (10 mg/kg) to spend a greater duration of time in the open arms of the plus maze, however this effect was not significant. High variability between litters may have lead to these non-significant findings, although the few studies examining anxiety-like behavior after fluoxetine treatment have provided conflicting results (Iniguez et al., 2010; Norcross et al., 2008; Oh, Zupan, Gross, & Toth, 2009). Iniguez et al.

(2010) and Oh et al. (2009) both found a decrease in entries and duration on the open arms of the elevated plus maze after long-term fluoxetine treatment. In contrast, Norcross et al. (2008) found no significant effects of fluoxetine treatment in this paradigm. The only study examining anxiety-like behavior after paroxetine treatment found a decrease in the time spent in the open arms (de Jong et al., 2006).

Sex-Differences in Markers of Depression and Suicide

To our knowledge, there is no published data on the behavioral effects of SSRI administration in adolescent females. Moreover, the studies conducted in adolescent male rats have produced mixed results. Overall, it seems that SSRIs do not affect or increase depression- and anxiety-like behaviors in adolescent rats (Olivier, Blom, Arentsen, & Homberg, 2010). In the present study, SSRI treatment did not affect the elevated plus maze performance of either male or female rats. The drugs had only a minimal effect on sucrose preference. The inconclusive results reported here and discrepancies in past research should provide further impetus to investigate the effects of SSRIs on adolescent behavior.

Only marginal sex differences in neurochemistry were induced by SSRI treatment. Sex differences in the serotonergic system of the prefrontal cortex tend to develop during puberty, which occurs around the fifth to sixth postnatal week (Ojeda & Urbanski, 1994). In the current study, SSRI treatment ended at the beginning of this stage of development. Therefore, the potential effect of sex-dependent hormones cannot be ignored. A sex-dependent effect was found in BDNF levels. Males did not show a significant response to fluoxetine treatment, while female rats had a dramatic increase in BDNF levels. Similarly, Hodes et al. (2009) demonstrated that fluoxetine increased hippocampal neurogenesis in females when compared to age-matched males. Fluoxetine treatment can significantly affect the regulation and the maturation of estrus cycling in rats (Amsterdam et al., 1997; Hodes et al., 2010; Maswood, Sarkar, & Uphouse, 2008). In particular, gonadal hormones can affect hippocampal neurogenesis (mediated by BDNF), possibly contributing to the observed sex differences in cell survival (Galea, 2008). Sex differences may also be due to variations in the metabolism of antidepressants. Compared to males, female rats sustain a higher level of norfluoxetine, the major active metabolite of fluoxetine (Ferguson & Hill,

2006). Higher circulating levels of norfluoxetine in females may increase serotonin levels for a longer period (Hodes et al., 2009). Thus, the pharmacokinetics of these SSRIs may play a role in the neurochemical differences between sexes (Hodes et al., 2010).

Conclusion

Caution is necessary when translating rodent data to humans, because there are important differences in metabolism, development, and hormones. However, there are many commonalities between suicidal adolescent humans and rats that exhibit behaviors associated with suicide. For example, in adolescent humans, plasma serotonin levels are negatively correlated with suicidal and violent behavior (Tyano et al., 2006), while 5HIAA levels are negatively correlated with aggression (Kruesi et al., 1990). Postmortem studies in adolescent humans have demonstrated an up-regulation of 5-HT_{2A} receptors and mRNA in the hippocampus and prefrontal cortex of suicide completers (Pandey et al., 2002). This indicates a reduction in serotonin availability in these particular brain regions. A similar pattern emerges in rodent studies using analogous age groups. Low levels of serotonin and serotonin turnover are correlated with high levels of

aggression (Eichelman & Thoa, 1973; Gibbons, Barr, Bridger, & Leibowitz, 1979; Miyachi, Kasai, Ohyama, & Yamada, 1994; Valzelli, 1973), as well as disinhibited behavior (Pothakos, Robinson, Gravanis, Marsteller, Dewey, & Tsirka, 2009). Results from the current study showed a decrease in serotonin after both SSRI treatments, but only fluoxetine reduced serotonin turnover. The current results suggest that short-term administration of paroxetine and fluoxetine may decrease serotonin activity in a manner similar to humans who exhibit suicidal behavior.

BDNF levels in depressed patients are negatively correlated with the severity of depression and suicidal behavior (Yoshimura et al., 2007). Furthermore, antidepressant treatment can increase BDNF levels in depressed patients (Aydemir, Deveci, & Taneli, 2005; Aydemir et al., 2006; Gervasoni et al., 2005; Gonul, Akdeniz, Taneli, Donat, Eker, & Vahip, 2005; Karege, Perret, Bondolfi, Schwald, Bertschy, & Aubry, 2002; Karege, Schwald, & Cisse, 2002; Shimizu et al., 2003). This human research suggests that BDNF levels may be a biological marker of suicidal depression (Yoshimura et al., 2007). Animal studies parallel these findings, demonstrating that reduced hippocampal BDNF levels can cause depressive-like behavior, while repeated

antidepressant treatment can cause neurogenesis by increasing BDNF (Wang, David, Monckton, Battaglia, & Hen, 2008). The present study found an increase in BDNF after fluoxetine treatment in females, which may imply that fluoxetine has a beneficial sex-specific effect during adolescence. Paroxetine on the other hand, caused no beneficial effect in females and had a negative consequence (e.g., decreased BDNF levels) in adolescent male rats. Administering paroxetine to adult humans affects BDNF levels in an inconsistent manner (Lee, Myint, & Kim, 2010); however, the majority of research shows that paroxetine exposure increases BDNF expression (Gonul et al., 2005; Hellweg, Ziegernorn, Heuser, & Deuschle, 2008; Yoshimura et al., 2007).

An important caveat is that these experiments were conducted using normal (i.e., non-depressed) rats. In other words, it is possible that our neurochemical results are not reflective of what would be observed using a "depressed" rat. However, a number of recent studies suggest that normal and "depressed" male rats respond similarly to SSRIs. Specifically, repeated SSRI treatment affects the brain neurochemistry, including BDNF expression, of non-stressed and stressed rats in a similar manner (chronic stress is a well-characterized rodent

model of depression) (Qi, Lin, Li, Wang, Wang, & Sun, 2008; Schulte-Herbrüggen, Fuchs, Ziegler, Danker-Hopfe, Hiemke, & Hellweg, 2009.

In summary, the current investigation showed that paroxetine administration during adolescence caused detrimental effects on neural mechanisms associated with suicidal and depressive behaviors. Importantly, the SSRI-induced changes in serotonin and BDNF did not affect depressive-like behavior as expected. The failure to find changes in our behavioral markers may have occurred because (a) the drug treatment did not extend long enough or (b) we did not wait long enough after conclusion of the drug regimen for the behavioral changes to manifest. While our work is preliminary, these data suggest that paroxetine may induce suicidal ideation and behavior by decreasing both serotonin and BDNF levels.

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