Published in final edited form as:

Psychopharmacology (Berl). 2015 October ; 232(19): 3515–3528. doi:10.1007/s00213-015-4003-1.

Effects of acute or repeated paroxetine and fluoxetine treatment on affective behavior in male and female adolescent rats

Leslie R. Amodeo, Venuz Y. Greenfield, Danielle E. Humphrey, Veronica Varela, Joseph A. Pipkin, Shannon E. Eaton, Jelesa D. Johnson, Christopher P. Plant, Zachary R. Harmony, Li Wang, and Cynthia A. Crawford

Department of Psychology, California State University, San Bernardino, CA, 92407, USA

Abstract

Rationale—The SSRI antidepressant fluoxetine is one of the few drugs that is effective at treating depression in adolescent humans. In contrast, the SSRI paroxetine has limited efficacy and is more at risk for inducing suicidal behavior.

Objective—The purpose of the present study was to more fully characterize the differential actions of paroxetine and fluoxetine.

Methods—In Experiment 1, male and female rats were injected with paroxetine (2.5 or 10 mg/kg), fluoxetine (10 mg/kg), or vehicle for 10 days starting on postnatal day (PD) 35, and affective behaviors were assessed using sucrose preference and elevated plus maze tasks. A separate set of rats were used to examine monoamine levels. In Experiment 2, rats were injected with paroxetine (2.5, 5 or 10 mg/kg), fluoxetine (5, 10 or 20 mg/kg), or vehicle during the same time frame as Experiment 1 and anxiety-like behaviors were measured using elevated plus maze, light/dark box, and acoustic startle.

Results—Repeated SSRI treatment failed to alter sucrose preference, although both paroxetine and fluoxetine reduced time spent in the open arms of the elevated plus maze and light compartment of the light/dark box. Paroxetine, but not fluoxetine, enhanced acoustic startle and interfered with habituation. Serotonin turnover was decreased by both acute and repeated fluoxetine treatment but unaltered by paroxetine administration.

Discussion—These results show that repeated treatment with paroxetine and fluoxetine has dissociable actions in adolescent rats. In particular, paroxetine, but not fluoxetine, increases acoustic startle at low doses and may increase sensitivity to environmental stressors.

Keywords

selective serotonin reuptake inhibitors (SSRI); adolescents; anhedonia; antidepressants; anxiety

*Correspondence to: Dr. Cynthia A. Crawford, Department of Psychology, California State University, San Bernardino, CA 92407. Phone: 909-537-7416. Fax: 909-537-7003. ccrawfor@csusb.edu.

Financial disclosure

The authors declare no conflict of interest.

Introduction

Major depressive disorder is one of the most commonly diagnosed psychiatric syndromes in the United States, with an estimated 12 month prevalence rate of 6.9% and a lifetime prevalence of 16.2 % (SAMHSA 2013; Hirschfeld 2014; Velehorsch et al. 2014). Moreover, one in five adults will experience a depressive episode during their lifetime (Kessler et al. 2003; Hirschfeld 2012). Depression is evident in younger populations as well, since the prevalence rate for adolescents is approximately 4 to 9.5% (Reinecke et al. 2009; Wagner et al. 2014). Both adults and adolescents can be debilitated by this disorder, because major depression impairs normal social interactions and decreases the ability to function in work or academic settings (Kessler et al. 2003; Richards 2011). Major depression also enhances the risk of suicide in adult and pediatric populations (Sher et al. 2010). Although major depression is more prevalent in adults, suicidal behaviors are more common in adolescents (Gvion and Apter 2012).

Given the prevalence and severity of major depressive disorder, it is not surprising that antidepressants are the most frequently prescribed medication in the United States for 18- to 44-year-olds, with one in ten people over the age of 12 using these drugs (Pratt et al. 2011). For adults, there is a large and diverse group of effective medications available for the treatment of depression (Gartlehner et al. 2011; Hirschfeld 2012), although the selective serotonin reuptake inhibitors (SSRIs) have become predominate over the last few decades (Pratt et al. 2011). The popularity of SSRIs stems from their safety and side-effect profiles, as well as their effectiveness at reducing symptoms of depression and anxiety (Cipriani et al. 2009).

In contrast to the multiple pharmacotherapies available for adult populations, only fluoxetine, an SSRI, has proven effective at treating depression in children and adolescents (Mann et al. 2006; Lovrin 2009; Masi et al. 2010; Hetrick et al. 2012). Another treatment complication affecting pediatric populations is that many antidepressants, including the SSRIs, increase suicidal ideation and behavior in adolescents and young adults (Emslie et al. 2006; Tiihonen et al. 2006; Lovrin 2009; Valluri et al. 2011). When compared to other SSRIs, fluoxetine is less likely to induce suicidal ideation; whereas, paroxetine is particularly prone to triggering suicidal behavior in adolescents (Tiihonen et al. 2006). As a consequence, the FDA, in 2003, recommended that paroxetine not be used with pediatric populations (Valluri et al. 2011).

The reason why fluoxetine and paroxetine differentially affect clinical efficacy and suicidal behavior of pediatric populations is not clear, especially since both compounds have similar mechanisms of action (i.e., they selectively block the serotonin transporter). Therefore, the goal of the present study was to assess the effects of repeated fluoxetine and paroxetine treatment on the depressive- and anxiety-like behaviors of male and female adolescent rats. To this end, we used the elevated plus maze, light/dark box and acoustic startle to measure anxiety-like behavior, while sucrose preference was used to measure anhedonia (an important component of clinical depression). Both sexes were tested in the present study because major depression is more prevalent in human females than males (Piccinelli and Wilkinson 2000; Parker and Brotchie 2010; Hirschfeld 2012). In addition, we measured

neuronal monoamine activity after adolescent SSRI exposure, since changes in serotonin utilization are associated with the beneficial effects of repeated SSRI treatment (Mikail et al. 2012; Nagano et al. 2012; Shishkina et al. 2012).

Material and methods

Subjects

A total of 877 male and female rats of Sprague-Dawley descent (Charles River Laboratories, Wilmington, MA), born and raised at California State University, San Bernardino (CSUSB), were used. Litters were culled to 10 pups on postnatal day (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. All animals were treated according to the “Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research” (National Research Council, 2010) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Drugs

Fluoxetine hydrochloride and paroxetine hydrochloride were obtained from Toronto Research Chemicals (Toronto, Canada). Both fluoxetine and paroxetine were dissolved in a 50% dimethyl sulfoxide (DMSO)/water solution and injected interperitoneally (IP) at a volume of 1 ml/kg.

Apparatus

The plus maze was made of black plastic and was elevated 50 cm above the floor (San Diego Instruments, San Diego, CA). The apparatus consisted of four arms, 50 cm long and 10 cm wide aligned perpendicularly. Two arms were enclosed by 30 cm high walls and the other two arms were exposed. The exposed or “open” arms had a 0.9 cm lip to help prevent rats from falling. The maze was placed in the center of a quiet, dimly lit room, with a digital video camera located above the maze. Acoustic startle response testing was conducted using the Coulbourn Animal Acoustic Startle System (Coulbourn Instruments, Whitehall, PA), which consisted of a weight-sensitive platform inside a sound-attenuating chamber. A ventilating fan built into the chamber provided background noise. The light/dark box consisted of a dark plastic box (20 × 20 × 40 cm), with a small transition door, that was fitted inside a clear Plexiglas photobeam chamber (40 × 40 × 40 cm, Coulbourn Instruments)

Procedures

Experiment 1: Acute and repeated effects of fluoxetine and paroxetine on body weight, sucrose preference, elevated plus maze performance, and monoamine content—On PD 31, rats ($n=10\text{--}11$ per group) began training on the sucrose preference task using a two-bottle choice test. On the first day, rats were singly housed and habituated to drinking water from two bottles. On the next three days (PD 32–34), one of the water bottles was replaced with a bottle containing a 2% sucrose solution. The bottles were weighed and refilled each day, and the position of the bottles was switched daily to avoid

position preferences. Rats in the repeated condition were returned to group housing where they were injected with paroxetine (2.5 or 10 mg/kg), fluoxetine (10 mg/kg) or vehicle once daily from PD 35 to PD 44; whereas, rats in the acute condition were group housed and injected with vehicle once daily from PD 35 to PD 43, and paroxetine (2.5 or 10 mg/kg), fluoxetine (10 mg/kg) or vehicle on PD 44. After the last drug injection, rats were returned to individual housing and a final sucrose test occurred 24 h later (i.e., on PD 45). Sucrose preference was defined using the following formula: $[(\text{weight of sucrose ingested}) / (\text{weight of water ingested} + \text{weight of sucrose ingested}) \times 100]$. A change in sucrose preference after SSRI treatment was assessed by calculating difference scores (i.e., the average of the three baseline preference scores minus the final preference score). Immediately following the sucrose test, rats were individually brought to a separate testing room and placed in the center of the elevated plus maze facing an open arm. Rats were left on the maze for 5 min. Time spent in the open and closed arms, as well as the center area, were scored from the videotapes. Entry into an open or closed arm was defined as all four paws crossing the threshold, whereas entry into the center of the maze was defined as two paws crossing the threshold. Separate groups of rats ($n = 10\text{--}11$ per groups) were used to assess preference for lower concentrations of sucrose (0.25 or 0.5%). Procedures were the same as described above.

A separate set of rats was used to assess the effects of repeated and acute SSRI treatment on various monoamine neurotransmitters and metabolites. Rats were killed 24 h after the last drug treatment and their hippocampus and prefrontal cortex removed and frozen. Frozen tissue samples were sonicated in 5 volumes of 0.1N HClO₄ and centrifuged at $20,000 \times g$ for 30 min at 4 °C. The supernatant was filtered through a 0.22 mm centrifugation unit at $2,000 \times g$ for 5 min at 4 °C. Twenty microliters of the resulting extracts were then assayed for norepinephrine, serotonin, and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) using high performance liquid chromatography with electrochemical detection (MD-150 column and Coulochem II detector; ESA, Chelmsford, MA). The mobile phase consisted of 75 mM NaH₂PO₄, 1.4 mM 1-octane sulfonic acid, 10 mM EDTA, and 7% acetonitrile [(pH 3.1) MD-TM Mobile Phase; ESA] and was pumped at a rate of 0.5 ml/min. The turnover rate of serotonin (5-HIAA/5-HT) was computed to assess serotonin utilization.

Experiment 2: Dose- and task-dependent differences in the effect of fluoxetine and paroxetine on elevated plus maze, light/dark box, and acoustic startle performance

performance—Rats ($n=9\text{--}11$ per group) received paroxetine (2.5, 5 or 10 mg/kg), fluoxetine (5, 10 or 20 mg/kg), or vehicle once daily from PD 35 to PD 44. On the following day (PD 45), rats were tested on an elevated plus maze, light/dark box, or in an acoustic startle chamber. Elevated plus maze performance was assessed using the same procedures as in Experiment 1, with the exception that videotapes were also scored for head dips. Rats tested in the light/dark box were individually placed in the light compartment to begin the 10-min test session. Time spent in each compartment and the frequency of transitions into the light compartment were measured. Rats in the acoustic startle/prepulse inhibition (PPI) experiment were placed into the testing chamber for a 5-min acclimation period prior to the delivery of any stimulus. The session was conducted using a 70 dB white noise background. On the first and last 6 trials of the session, a startling stimulus [50 dB above background (or

120 dB), 40 ms] was presented alone. The middle 32 trials were presented in a pseudorandom order and included 12 stimulus-alone trials (used to calculate % PPI and average startle amplitude), and 12 prepulse trials in which a prepulse stimulus (20 ms) preceded the startling stimulus by 100 ms. The prepulse stimuli were 3, 6, 12, 15 or 18 dB above background. Additionally, there were 8 trials on which no stimulus was presented. The intertrial interval was 20 s. Startle amplitude during the 12 stimulus-alone trials was averaged and was used in the analysis of prepulse inhibition. Percent prepulse inhibition was calculated as $100 \times (\text{average startle amplitude on the prepulse trials} / \text{average startle amplitude on the stimulus-alone trials})$. Percent habituation was calculated as $100 \times (\text{average startle amplitude on the last 6 startle-alone trials} / \text{average startle amplitude on the first 6 startle-alone trials})$.

Data analysis

Body weights during the injection phase were analyzed using a pretreatment condition $\times$ sex $\times$ day repeated measures ANOVA. When the assumption of sphericity was violated, as determined by Mauchly's test of sphericity, the Greenhouse-Geisser epsilon statistic was used to adjust degrees of freedom (Geisser and Greenhouse 1958). Corrected degrees of freedom were rounded to the nearest whole number. Data from the sucrose preference task, elevated plus maze, and monoamine assays were analyzed using pretreatment $\times$ sex ANOVAs. For the monoamine assays, separate statistical analyses were conducted for the two brain regions (hippocampus and prefrontal cortex). For Experiment 2, paroxetine and fluoxetine data were analyzed by separate ANOVAs. Dunnett post hoc tests were used to compare differences between the drug and vehicle groups, while all other pairwise comparisons were computed using the Benjamini-Hochberg correction for False Discovery Rate (FDR). For all statistical tests, the significance level was set at $P < 0.05$. To control for litter effects, no more than one subject per litter was assigned to a particular group. In situations where this was not possible, a single litter mean was calculated from multiple littermates assigned to the same group (Holson and Pearce 1992; Zorrilla 1997).

Results

Experiment 1: Acute and repeated effects of fluoxetine and paroxetine on sucrose preference, elevated plus maze performance, and monoamine content

Body weight—During the injection period (i.e., PD 35–44), male and female rats exhibited a progressive increase in body weight, with male rats weighing more than females on each day (Fig. 1) [day main effect, $F_{4,565}=1768.50$, $P < 0.001$; day $\times$ sex interaction, $F_{4,565}=130.46$, $P < 0.001$]. The increase in body weight was altered by SSRI treatment [day $\times$ drug $\times$ sex interaction, $F_{12,565}=4.21$, $P < 0.001$]. Relative to vehicle controls, fluoxetine (10 mg/kg) reduced the body weights of female rats on PD 39, PD 40, and PD 42–44, while decreasing the body weights of male rats on PD 40–44 (Dunnett tests, $P < 0.05$). Paroxetine (10 mg/kg) also reduced the body weights of male rats on PD 41–44, but did not significantly affect the weights of female rats. The lower dose of paroxetine (2.5 mg/kg) did not alter body weights of male or female rats. Acutely administering paroxetine (2.5 or 10 mg/kg) or fluoxetine (10 mg/kg) on PD 44 also did not affect body weights (data not shown).

Sucrose consumption and preference—Acute treatment with fluoxetine decreased both sucrose consumption and preference for a 2% sucrose solution on the test day [drug main effect, $F_{3,85}=6.13$, $P<0.001$; $F_{3,85}=2.91$, $P<0.05$; respectively; FDR (corrected $P<0.017$)] (Table 1). Acute paroxetine administration did not alter either sucrose measures. In addition, male rats consumed greater amounts of sucrose and had larger preferences than female rats [sex main effect, $F_{1,85}=4.79$, $P<0.05$; $F_{1,85}=6.25$, $P<0.05$; respectively]. When given repeatedly, exposure to paroxetine or fluoxetine did not alter preference for a 2% sucrose solution (Table 2). This was the case regardless of whether sucrose preference data were analyzed as absolute values or as difference scores (i.e., test day values relative to baseline). There were also no sex- or drug-dependent differences in 2% sucrose consumption. Rats tested with lower concentrations of sucrose also failed to show a drug-dependent difference in sucrose preference (data not shown). However, rats given the 0.5% sucrose solution consumed more sucrose ($M=58.35$ g, $SEM\pm 4.06$) and had a greater preference ($M=84.98\%$, $SEM\pm 2.7$) than rats given the 0.25% solution ($M=23.43$ g, $SEM\pm 1.31$; $M=61.80\%$, $SEM\pm 2.61$) [sucrose concentration main effect, $F_{1,156}=66.01$, $P<0.001$; $F_{1,156}=34.72$, $P<0.001$; respectively]. In addition, male rats ($M=46.19$ g, $SEM\pm 3.95$) consumed more sucrose than female rats ($M=35.63$ g, $SEM\pm 3.01$) [sex main effect, $F_{1,156}=6.36$, $P<0.05$]

Elevated plus maze—Acute administration of paroxetine and fluoxetine did not alter behavior on the elevated plus maze (data not shown). However, female rats ($M=51.19\%$, $SEM\pm 1.5$) spent more time in the open arms of the elevated plus maze than similarly treated male rats ($M=43.94\%$, $SEM\pm 2.5$) [sex main effect, $F_{3,88}=6.51$, $P<0.05$]. Repeated exposure to a moderate dose of paroxetine increased the anxiety of male and female rats, because rats treated with 10 mg/kg paroxetine spent less time in the open arms of the elevated plus maze than rats treated with vehicle or 2.5 mg/kg paroxetine (Fig. 2) [drug main effect, $F_{3,90}=4.33$, $P<0.01$; FDR (corrected $P<0.017$)]. Fluoxetine did not affect time spent in the open arms of the elevated plus maze.

Monoamine content—Treating rats repeatedly with paroxetine (10 mg/kg) on PD 35–44 reduced hippocampal serotonin levels, but not norepinephrine levels, when measured on PD 45 (Table 3) [drug main effect, $F_{3,48}=3.29$, $P<0.05$; $F_{3,48}=1.62$, $P=0.19$, respectively; Dunnett tests, $P<0.05$]. Fluoxetine did not affect serotonin or norepinephrine levels in the hippocampus. Both paroxetine and fluoxetine decreased 5-HIAA content in the hippocampus [drug main effect, $F_{3,48}=13.81$, $P<0.001$; Dunnett tests, $P<0.05$]; however, paroxetine-induced reductions in 5-HIAA levels were primarily driven by changes in female rats [sex $\times$ drug interaction, $F_{3,48}=3.48$, $P<0.05$; FDR (corrected $P<0.025$)]. Serotonin turnover (i.e., 5-HIAA/5-HT) was also differentially affected by fluoxetine and paroxetine treatment, because only fluoxetine reduced serotonin metabolism in the hippocampus [drug main effect, $F_{3,48}=6.40$, $P<0.001$; Dunnett tests, $P<0.05$].

In the prefrontal cortex, serotonin levels were reduced after repeated treatment with either paroxetine (10 mg/kg) or fluoxetine (10 mg/kg) (Table 4) [drug main effect, $F_{3,48}=7.82$, $P<0.001$; Dunnett tests, $P<0.05$]. In contrast, there was a trend for norepinephrine levels in the prefrontal cortex to be elevated after repeated fluoxetine treatment, but this slight

elevation was only evident in male rats [drug $\times$ sex interaction, $F_{3,48}=3.54$, $P<0.05$; FDR (corrected $P<0.008$; comparison was not significant)]. Paroxetine treatment did not increase norepinephrine levels in the prefrontal cortex. Similar to what was observed in the hippocampus, 5-HIAA content and serotonin turnover in the prefrontal cortex were reduced by fluoxetine, but not paroxetine [drug main effect, $F_{3,48}=3.79$, $P<0.05$; $F_{3,48}=3.80$, $P<0.05$, respectively; Dunnett tests, $P<0.05$].

Acute treatment with paroxetine and fluoxetine produced a different pattern of results than did repeated treatment (Tables 5 and 6). Most notably, neither compound significantly altered serotonin, 5-HIAA, or norepinephrine levels in the hippocampus or prefrontal cortex, although 10 mg/kg paroxetine marginally enhanced norepinephrine levels in the latter brain region [drug main effect, $F_{3,56}=2.56$, $P=0.06$]. Interestingly, acute treatment with fluoxetine, but not paroxetine, decreased serotonin turnover in the hippocampus (Table 4) [drug main effect, $F_{3,56}=4.97$; $P<0.05$; Dunnett tests, $P<0.05$]. A similar effect was observed after repeated fluoxetine treatment.

Experiment 2: Dose- and task-dependent differences in the effect of fluoxetine and paroxetine on elevated plus maze, light/dark box, and acoustic startle performance

Body Weight—In both the fluoxetine and paroxetine experiments, the body weights of male and female rats increased progressively across days, with male rats weighing more than females on each day (Fig. 3) [day main effect, $F_{4,231}=1232.89$, $P<0.001$; $F_{4,271}=617.56$, $P<0.001$; day $\times$ sex interaction, $F_{4,231}=88.26$, $P<0.001$; $F_{4,271}=40.98$, $P<0.001$]. The increase in body weight was altered by SSRI treatment [day $\times$ drug $\times$ sex interaction, $F_{11,231}=3.57$, $P<0.001$; $F_{13,271}=4.42$, $P<0.001$]. Paroxetine (10 mg/kg) reduced the body weights of male rats on PD 41–44, but did not significantly affect the weights of female rats. Lower doses of paroxetine (2.5 and 5 mg/kg) and fluoxetine (5 mg/kg) did not alter body weights of male or female rats. The highest dose of fluoxetine (20 mg/kg) decreased the body weight of females rats from PD 36–PD 44 and male rats from PD 37–PD 44 (Dunnett tests, $P<0.05$). In contrast to Experiment 1, 10 mg/kg fluoxetine produced a sex-dependent effect, because male rats exhibited lower body weights on PD 40–44 while, female rats did not show a significant change in body weight (Dunnett tests, $P<0.05$).

Elevated Plus Maze—Similar to Experiment 1, rats treated with paroxetine (10 mg/kg) spent less time in the open arms of the elevated plus maze than vehicle-treated rats [drug main effect, $F_{3,68}=4.78$, $P<0.01$; Dunnett tests, $P<0.05$]. Paroxetine treatment did not alter the number of entries in the closed arms (measure of general activity) or head dips (see Fig. 4, left panel). The highest dose of fluoxetine (20 mg/kg) also decreased time spent in the open arms of the elevated plus maze (see Fig. 4, right panel) [drug main effect, $F_{3,69}=6.04$, $P<0.01$; Dunnett tests, $P<0.05$], while significantly reducing head dips and closed arm entries [drug main effect, $F_{3,69}=3.20$, $P<0.05$; $F_{3,69}=4.43$, $P<0.01$, respectively; Dunnett tests, $P<0.05$]. The lower doses of fluoxetine did not alter elevated plus maze performance. Neither drug caused a sex-dependent effect.

Light/Dark Box—Repeated paroxetine treatment (5 mg/kg) reduced the duration of time spent in the light compartment relative to vehicle-treated rats (see Fig. 5, left panel) [drug

main effect, $F_{3,70}=4.32$, $P<0.01$; Dunnett tests, $P<0.05$]. The higher dose of paroxetine (10 mg/kg) marginally reduced duration in the light compartment, however this effect was not statistically significant ($P=0.08$). Neither paroxetine treatment nor sex altered the number of light/dark transitions. Repeated fluoxetine treatment had a similar effect on behavior in the light/dark box, because rats treated with 10 or 20 mg/kg fluoxetine spent less time in the light compartment than vehicle controls (see Fig. 5, right panel) [drug main effect, $F_{3,68}=10.24$, $P<0.001$; Dunnett tests, $P<0.05$]. There were no fluoxetine-induced differences in light/dark transitions.

Acoustic Startle—Regardless of drug treatment, startle magnitude was greater in male rats than female rats (see Fig. 6) [sex main effect, $F_{1,79}=10.57$, $P<0.01$; $F_{1,73}=9.21$, $P<0.01$; respectively]. The lowest dose of paroxetine (2.5 mg/kg) increased startle magnitude [drug main effect, $F_{1,79}=10.57$, $P<0.01$; Dunnett tests, $P<0.05$], while no dose of fluoxetine significantly altered this behavior.

Startle habituation also differed according to sex. Specifically, male rats treated with fluoxetine exhibited less habituation from block 1 to block 2 (i.e., they had greater percent habituation scores) than female rats [sex main effect, $F_{1,73}=12.11$, $P<0.01$]. In the paroxetine experiment, vehicle-treated male rats had greater percent habituation scores than similarly treated female rats [sex $\times$ drug interaction, $F_{3,79}=6.10$, $P<0.001$; FDR (corrected $P<0.012$)]. Repeated treatment with 2.5 mg/kg paroxetine increased startle habituation, but only in female rats (FDR, corrected $P<0.025$). Interestingly, male rats displayed a decrease in startle habituation after 10 mg/kg paroxetine treatment (FDR, corrected $P<0.025$). Neither sex nor SSRI treatment altered PPI scores (data not shown).

Discussion

In adolescent humans, the antidepressants fluoxetine and paroxetine differ in their clinical efficacy and risk of suicidal ideation; with a preponderance of evidence showing that fluoxetine is an effective treatment for pediatric populations, while the efficacy and safety of paroxetine is in question (Tiihonen et al. 2006; Masi et al. 2010; Valluri et al. 2010; Hetrick et al. 2012). The reason for the decreased treatment efficacy and increased risk for suicidal behavior in pediatric populations is unknown, but the present study suggests that the two SSRIs differ in their ability to affect certain anxiety-like behaviors and serotonin metabolism. Specifically, we found that both repeated paroxetine (10 and 5 mg/kg) and fluoxetine (20 mg/kg) treatment decreased time spent in the open arms of the elevated plus maze and the light side of the light/dark box, but only paroxetine (2.5 mg/kg) enhanced acoustic startle magnitude and altered habituation to acoustic startle. In addition, paroxetine treatment significantly reduced serotonin content in the prefrontal cortex and hippocampus, but did not alter serotonin turnover in either brain area. In contrast, repeated fluoxetine treatment reduced serotonin content in the prefrontal cortex, and decreased serotonin turnover in the prefrontal cortex and hippocampus.

Sucrose preference is an often used measure of anhedonia, and the ability of a drug to enhance sucrose preference is indicative of antidepressant actions (Strekalova et al. 2011). In the present study, preference for a 0.125–2% sucrose solution was not affected by

repeated fluoxetine or paroxetine treatment, since the sucrose consumption of SSRI-treated rats did not differ from vehicle-treated rats or from baseline preference scores. In a previous investigation, chronically (e.g., 15 days) treating adolescent male rats with fluoxetine (10 mg/kg) caused a small, but significant, increase in the preference for a low concentration (0.25%) of sucrose when tested 24 h after the last drug treatment (Iñiguez et al. 2010). It is unclear what accounts for the discrepancy in results, but it is possible that procedural differences (i.e., number and frequency of drug administration) were the cause. In any case, there was no evidence in the present study that administering fluoxetine or paroxetine during adolescence causes anhedonia after a 24 h washout period.

In contrast to our measure of anhedonia, the anxiety-like behaviors of male and female rats, as measured by performance on the elevated plus maze and the light/dark box, was altered by SSRI treatment. Specifically, treatment with paroxetine (10 mg/kg) or fluoxetine (20 mg/kg), increased anxiety-like behavior on the elevated plus maze (i.e., rats spent less time in the open arms). Acute exposure to paroxetine or fluoxetine reliably induces anxiogenic behavior in adult rodents (Kurt et al. 2000; Silva and Brandão 2000; Drapier et al. 2007; Liu et al. 2010); however, the effects of repeated SSRI treatment are less clear. For fluoxetine, the effects of repeated treatment appear to be age-dependent, because exposing preweanling rats to fluoxetine increases anxiety (Oh et al. 2009). In contrast, repeatedly administering fluoxetine to adult rats either decreases or has no effect on anxiety (Silva and Brandão 2000; Oh et al. 2009; but see Homberg et al. 2011). Adolescent exposure to fluoxetine appears to have mixed effects on anxiety-like behavior, with the time interval between testing and drug administration being a critical determining factor. If testing occurs during drug administration or after a very short washout interval, then fluoxetine increases anxiety-like behavior (Oh et al. 2009; Iñiguez et al. 2010); however, when testing occurs after a prolonged washout interval, then anxiety-like behavior is either not altered or decreases (Norcross et al. 2008; Oh et al. 2009; Vorhees et al. 2011; but see Iñiguez et al. 2010; Homberg et al. 2011). In the present study, we also found that fluoxetine reduced time spent in the open arms of the elevated plus maze (i.e., it enhanced anxiety-like behavior) after a 24 h washout. However, because fluoxetine (20 mg/kg) also decreased the number of entries into the closed arms (a measure of general activity) it is possible that the decreased time spent in the open arms was partially due to a drug-induced decrease in activity. Less is known about how chronic paroxetine affects anxiety-like behaviors, but it appears that repeated treatment during adolescence reduces time in the open arms of the elevated plus maze when measured after a prolonged washout period (de Jong et al. 2006; but see Vorhees et al. 2011). Handling effects could also have contributed to the increased anxiety we found after fluoxetine and paroxetine treatment, because in our first experiment we used the same rats for both the sucrose preference and elevated plus maze and the rats were given their last drug injection 24 h before behavioral testing began. Thus, it is possible that the stress of our testing procedures increased anxiety-like behavior (i.e., decreased time in the open arms), however this effect could not solely account for the differences between our control and drug-treated rats.

Similar to results gained using the elevated plus maze, repeated paroxetine (5 mg/kg) and fluoxetine (10 and 20 mg/kg) treatment increased the anxiety-like behavior of adolescent rats when measured using the light/dark box (i.e., rats spent less time in the light

compartment). Consistent with this finding, Majidi-Zolbanin et al. (2013) reported that male mice treated with fluoxetine from PD 35-PD 65 spent less time in the light compartment than vehicle-treated mice. To our knowledge, no previous studies have assessed the effects of repeated paroxetine treatment on the performance adolescent rats in the light/dark box, but it appears that like fluoxetine, repeated paroxetine treatment decreases time spent in the light compartment. In general, results gained using adult rodents are less consistent, because acute fluoxetine treatment has been reported to increase (de Angelis 1996), decrease (Birkett et al. 2011; Arrant et al. 2013), or have no effect on time spent in the light compartment (Emmanouil et al. 2006). Repeated treatment with fluoxetine also produced varied outcomes in adult rodents depending on the length of drug exposure. Specifically, subchronic (under 20 days) exposure to fluoxetine has no effect on time spent in the light compartment (Ihne et al. 2012; Lesemann et al. 2012), while chronic (over 20 days) exposure has been reported to both increase (Ihne et al. 2012) and decrease time spent in the light compartment of the light/dark box (Vicente and Zangrossi 2014). Curiously, acute and chronic (e.g. 28 days) treatment with paroxetine decreases anxiety-like behavior in adult rodents (Hascoët et al. 2000; Sillaber et al. 2008). Age-dependent differences in the action of paroxetine are of potential importance because SSRI-induced increases in anxiety-like behavior raise the risk of suicidal behavior in adolescent humans (Hawgood and De Leo 2008; Sareen 2011).

Interestingly, unlike our previous measures of anxiety, startle magnitude and habituation were differentially modulated by paroxetine and fluoxetine treatment. Specifically, repeated treatment with paroxetine (2.5 mg/kg), but not fluoxetine, increased startle magnitude in male and female rats. Moreover, this dose of paroxetine also decreased the rate of habituation in female rats. Curiously, however, male rats did not show this decrease in habituation rates and actually had greater habituation after a high dose of paroxetine (10 mg/kg). Although there is not a large literature assessing the effects of SSRI on acoustic startle, adult rats treated repeatedly with SSRIs from two to three weeks generally show a decrease in acoustic startle (Fujiwara et al. 2011; Homberg et al. 2011; Miles et al. 2011). In contrast, repeated (e.g., 20 days) fluoxetine treatment has been reported to either increase (Vorhees et al. 2011) or have no effect on the startle magnitude of adolescent rats (Homberg et al. 2011; present study). Repeatedly administering paroxetine during adolescence has also been reported to either increase startle magnitude (Vorhees et al. 2011) or have no effect (de Jong et al. 2012). Interestingly, Vorhees et al. (2011) found that paroxetine had a greater effect on acoustic startle than fluoxetine. This pattern of results is consistent with our findings, and suggests that paroxetine may have a more robust effect on acoustic startle than fluoxetine. Given that an exaggerated startle response is positively correlated with anxiety-like behaviors in both animals and humans (Frankland et al. 1997; Davis 1998; McMillan et al. 2012) and is a core symptom in PTSD (Grillon et al. 1996), the ability of paroxetine to augment startle is consistent with its lack of efficacy in adolescent humans.

Both SSRIs affected the body weights of adolescent rats. Repeated fluoxetine treatment reduced body weight gain, relative to vehicle controls, in both males and females. This decrease in body weight is consistent with past studies showing that fluoxetine reduces body weights of adult and adolescent rodents as well as humans (Mitchell et al. 2003; McNamara et al. 2010; Serretti and Mandelli 2010; Vorhees et al. 2011; Guirado et al. 2012). Repeated treatment with paroxetine (10 mg/kg) attenuated daily weight gain in adolescent male rats,

but not female rats. The reason for this sex difference is uncertain, but it may be a consequence of drug pharmacokinetics. Other researchers have also reported that short-term (5–15 days) paroxetine treatment decreases the body weight of rodents (Chen et al. 2004; de Jong et al. 2006); however, human data indicates that chronic paroxetine treatment (26 to 32 weeks) leads to an increase in body weight (Fava et al. 2000; Kim et al. 2006; Nelson et al. 2006).

In adult rodents, repeated exposure to SSRIs decreases serotonin turnover in the hippocampus and prefrontal cortex (Baldessarini et al. 1992; Frankfurt et al. 1994; Harkin et al. 2003; Thompson et al. 2004; Miura et al. 2007; Durkin et al. 2008). This action is believed to be a consequence of SSRIs inhibiting serotonin reuptake and metabolism (Baldessarini et al. 1992; Frankfurt et al. 1994; Leonardi and Azmitia 1994; Harkin et al. 2003; Thompson et al. 2004). Interestingly, we found that fluoxetine, but not paroxetine, decreased serotonin turnover in both the hippocampus and prefrontal cortex of adolescent rats. Because decreased serotonin turnover is positively correlated with the ability to reduce depressive- and anxiety-like behaviors (Thompson et al. 2004; Shishkina et al. 2012), this result may explain paroxetine's lack of clinical efficacy. In addition, we found that both paroxetine (10 mg/kg) and fluoxetine reduced serotonin levels in the prefrontal cortex, while only paroxetine decreased serotonin content in the hippocampus. Repeated SSRI treatment has occasionally been shown to reduce serotonin content in various brain regions of adult rodents (Caccia et al. 1992; Trouvin et al. 1993; Thompson et al. 2004), although nonsignificant effects are more typically reported (Harkin et al. 2003; Durkin et al. 2008; Karanges et al. 2011; Shishkina et al. 2012). SSRI-induced reductions in serotonin content are usually observed after extended drug exposure (i.e., 3 or more weeks) or when high doses of SSRI are administered. In the present study, brain serotonin levels declined after administering moderate doses (i.e., 10 mg/kg once a day) of fluoxetine and paroxetine for a relatively short 10-day period, thus suggesting that juvenile rats may be more sensitive to the serotonin-depleting effects of SSRI treatment. If similar changes in sensitivity occur in humans, the efficacy of SSRIs during adolescence could be reduced.

Conclusion

In summary, repeated treatment with fluoxetine and paroxetine produces different behavioral and neurochemical responses in adolescent rats. Specifically, both paroxetine and fluoxetine exposure increased anxiety-like behaviors on the elevated plus maze and light/dark box, but only paroxetine altered acoustic startle. Also, paroxetine had no effect on serotonin turnover, while fluoxetine produced an adult-like decline in turnover. This pattern of results shows that short-term exposure to paroxetine and fluoxetine produces idiosyncratic effects that may be responsible for the observed differences in clinical efficacy. These effects may be dependent on the length of drug exposure, therefore additional studies using longer (> 3 weeks) exposure periods are warranted. It will also be important to investigate the effects of adolescent SSRI exposure using a validated animal model of depression (e.g., maternal separation or chronic mild stress).

Acknowledgments

This work was supported by NIGMS training grant GM083883 (VR and JDJ), GM100829 (CPP) and NIDA training grant DA025319 (JAP)

REFERENCES

- Arrant AE, Coburn E, Jacobsen J, Kuhn CM. Lower anxiogenic effects of serotonin agonists are associated with lower activation of amygdala and lateral orbital cortex in adolescent male rats. *Neuropharmacology*. 2013; 73:359–367. [PubMed: 23774134]
- Baldessarini RJ, Marsh ER, Kula NS. Interactions of fluoxetine with metabolism of dopamine and serotonin in rat brain regions. *Brain Res*. 1992; 579:152–156. [PubMed: 1623401]
- Birkett MA, Shinday NM, Kessler EJ, Meyer JS, Ritchie S, Rowlett JK. Acute anxiogenic-like effects of selective serotonin reuptake inhibitors are attenuated by benzodiazepine diazepam in BALB/c mice. *Pharmacol Biochem Behav*. 2011; 98:544–551. [PubMed: 21397628]
- Caccia S, Fracasso C, Garatini S, Guiso G, Sarati S. Effects of short- and long-term administration of fluoxetine on the monoamine content of rat brain. *Neuropharmacology*. 1992; 31:343–347. [PubMed: 1381817]
- Chen Z, Waimey K, Van de Kar L, Carrasco GA, Landry M, Battaglia G. Prenatal cocaine exposure potentiates paroxetine-induced desensitization of 5-HT_{2A} receptor function in adult male rat offspring. *Neuropharmacology*. 2004; 46:942–953. [PubMed: 15081791]
- Cipriani A, Furukawa A, Salanti G, Geddes JR, Higgins JPT, Churchill R, Watanabe N, Nakagawa A, Omori IM, McGuire H, Tansella M, Barbui C. Comparative and acceptability of 12 new generation antidepressants: a multiple-treatments meta-analysis. *Lancet*. 2009; 373:746–758. [PubMed: 19185342]
- Davis M. Anatomic and physiologic substrates of emotion in an animal model. *J Clin Neurophysiol*. 1998; 15:378–387. [PubMed: 9821065]
- De Angelis L. Experimental anxiety and antidepressant drugs: the effects of moclobemide, a selective reversible MAO-A inhibitor, fluoxetine and imipramine in mice. *Naunyn Schmiedeberg Arch Pharmacol*. 1996; 354:379–383. [PubMed: 8878071]
- de Jong TR, Snaphaan JAE, Pattij T, Veening JG, Waldinger MD, Cools AR, Olivier B. Effects of chronic treatment with fluvoxamine and paroxetine during adolescence on serotonin-related behavior in adult male rats. *Eur Neuropsychopharmacol*. 2006; 16:39–48.
- Drapier D, Bentué-Ferrer D, Laviolle B, Millet B, Allain H, Bourin M, Reymann JM. Effects of acute fluoxetine, paroxetine and desipramine on rats tested on the elevated plus-maze. *Behav Brain Res*. 2007; 176:202–209. [PubMed: 17095104]
- Durkin S, Prendergast A, Harkin A. Reduced efficacy of fluoxetine following MDMA (“ecstasy”)-induced serotonin loss in rats. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008; 32:1894–1901. [PubMed: 18824064]
- Emmanouil DE, Papadopoulou-Daifoti Z, Hagihara PT, Quock DG, Quock RM. A study of the role of serotonin in the anxiolytic effect of nitrous oxide in rodents. *Pharmacol Biochem Behav*. 2006; 84:313–320. [PubMed: 16828854]
- Emslie GJ, Wagner KD, Kutcher S, Krulwicz S, Fong R, Carpenter DJ, Lipschitz A, Machin A, Wilkinson C. Paroxetine treatment in children and adolescents with major depressive disorder: a randomized, multicenter, double-blind, placebo-controlled trial. *J Am Acad Child Adolesc Psychiatry*. 2006; 45:709–719. [PubMed: 16721321]
- Fava M, Judge R, Hoog SL, Nilsson ME, Koke SC. Fluoxetine versus sertraline and paroxetine in major depressive disorder: changes in weight with long-term treatment. *J Clin Psychiatry*. 2000; 61:863–867. [PubMed: 11105740]
- Frankfurt M, McKittrick CR, Luine VN. Short-term fluoxetine treatment alters monoamine levels and turnover in discrete brain nuclei. *Brain Res*. 1994; 650:127–132. [PubMed: 7525014]
- Frankland PW, Josselyn SA, Bradwejn J, Vaccariono FJ, Yeomans JS. Activation of amygdala cholecystokinergic receptors potentiates the acoustic startle response in the rat. *J Neurosci*. 1997; 17:1838–1847. [PubMed: 9030642]

- Fujiwara K, Asakura M, Yanagida T, Nakano M, Kanai S, Tanaka D, Susuga Y, Osada K. The delayed sensitization of CRH response developed after chronic variable stress on the acoustic startle reflex. *Nihon Shinkei Seishin Yakurigaku Zasshi*. 2011; 31:17–22. [PubMed: 21409840]
- Gartlehner G, Hansen RA, Morgan LC, Thaler K, Lux L, Van Noord M, Mager U, Thieda P, Gaynes BN, Wilkins T, Strobelberger M, Lloyd S, Reichenpfader U, Lohr KN. Comparative benefits and harms of second-generation antidepressants for treating major depressive disorder. *Ann Intern Med*. 2011; 155:772–785. [PubMed: 22147715]
- Geisser S, Greenhouse SW. An extension of Box's results on the use of the *F* distribution in multivariate analysis. *Ann Mat Statist*. 1958; 29:885–891.
- Grillon C, Morgan CA, Southwick SM, Davis M, Charney DS. Baseline startle amplitude and prepulse inhibition in Vietnam veterans with posttraumatic stress disorder. *Psychiatry Res*. 1996; 64:169–178. [PubMed: 8944395]
- Guirado R, Sanchez-Matarredona D, Vereja E, Crespo C, Blasco-Ibáñez JM, Nacher J. Chronic fluoxetine treatment in middle-aged rats induces changes in the expression of plasticity-related molecules and in neurogenesis. *BMC Neurosci*. 2012; 13:5. [PubMed: 22221403]
- Gvion Y, Apter A. Suicide and suicidal behavior. *Public Health Rev*. 2012; 34:1–20. [PubMed: 26236074]
- Harkin A, Shanahan E, Kelley JP, Connor TJ. Methylenedioxymphetamine produces serotonin nerve terminal loss and diminished behavioural and neurochemical responses to the antidepressant fluoxetine. *Eur J Neurosci*. 2003; 18:1021–1027. [PubMed: 12925028]
- Hascoët M, Bourin M, Nic Dhonnchada BA. The influence of buspirone, and its metabolite 1-PP, on the activity of paroxetine in the mouse light/dark paradigm and four plates test. *Pharmacol Biochem Behav*. 2000; 67:45–53. [PubMed: 11113483]
- Hawgood J, De Leo D. Anxiety disorders and suicidal behavior: an update. *Curr Opin Psychiatry*. 2008; 21:51–64. [PubMed: 18281841]
- Hirschfeld MA. The epidemiology of depression and the evolution of treatment. *J Clin Psychiatry*. 2012; 73(suppl 1):5–9. [PubMed: 22951236]
- Hirschfeld MA. Differential diagnosis of bipolar and major depressive disorder. *J Affect Disord*. 2014; 151:S12–S16. [PubMed: 25533909]
- Hetrick SE, McKenzie JE, Cox GR, Simmons MB, Merry SN. Newer generation antidepressants for depressive disorders in children and adolescents. *Cochrane Database Syst Rev*. 2012; 11:004851.
- Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol Teratol*. 1992; 14:221–228. [PubMed: 1635542]
- Homberg JR, Olivier JD, Blom T, Arentsen T, van Brunschot C, Schipper P, Korte-Bouws G, van Luijckelaar G, Reneman L. Fluoxetine exerts age-dependent effects on behavior and amygdala neuroplasticity in the rat. *PLoS One*. 2011; 6:16646.
- Ilhne JL, Fitzgerald PJ, Hefner KR, Holmes A. Pharmacological modulation of stress-induced behavioral changes in the light/dark exploration test in male C57BL/6J mice. *Neuropharmacology*. 2012; 62:464–473. [PubMed: 21906605]
- Iñiguez SD, Warren BL, Bolaños-Guzmán CA. Short- and long-term functional consequences of fluoxetine exposure during adolescence in male rats. *Biol Psychiatry*. 2010; 67:1057–1066. [PubMed: 20172503]
- Karanges E, Li KM, Motbey C, Callaghan PD, Katsifis A, McGregor IS. Differential behavioural and neurochemical outcomes from chronic paroxetine treatment in adolescent and adult rats: a model of adverse antidepressant effects in human adolescents? *Int J Neuropsychopharmacol*. 2011; 14:491–504. [PubMed: 21329552]
- Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R) JAMA. 2003; 289:3095–3105. [PubMed: 12813115]
- Kim T-S, Pae C-U, Yoon S-J, Bahk W-M, Jun T-Y, Rhee W-I, Chae J-H. Comparison of venlafaxine extended release versus paroxetine for treatment of patients with generalized anxiety disorder. *Psychiatry Clin Neurosci*. 2006; 60:347–351. [PubMed: 16732752]
- Kurt M, Arik AC, Celik S. The effects of sertraline and fluoxetine on anxiety in the elevated plus-maze in mice. *J Basic Clin Physiol Pharmacol*. 2000; 11:173–180. [PubMed: 11037770]

- Leonardi ET, Azmitia EC. MDMA (ecstasy) inhibition of MAO type A and type B: comparisons with fenfluramine and fluoxetine (Prozac). *Neuropsychopharmacology*. 1994; 10:231–238. [PubMed: 7945733]
- Lesemann A, Reinel C, Hühnchen P, Pilhatsch M, Hellweg R, Klaissler P, Winter C, Steiner B. MPTP-induced hippocampal effects on serotonin, dopamine, neurotrophins, adult neurogenesis and depression-like behavior are partially influenced by fluoxetine in adult mice. *Brain Res*. 2012; 1457:51–69. [PubMed: 22520437]
- Liu J, Garza JC, Bronner J, Kim CS, Zhang W, Lu XY. Acute administration of leptin produces anxiolytic-like effects: a comparison with fluoxetine. *Psychopharmacology (Berl)*. 2010; 207:535–545. [PubMed: 19823809]
- Lovrin M. Treatment of major depression in adolescents: weighing the evidence of risk and benefit in light of black box warnings. *J Child Adolesc Psychiatr Nurs*. 2009; 22:63–68. [PubMed: 19490276]
- Majidi-Zolbanin J, Azarfarin M, Samadi H, Enayati M, Salari A-A. Adolescent fluoxetine treatment decreases the effects of neonatal immune activation on anxiety-like behavior in mice. *Behav Brain Res*. 2013; 250:123–132. [PubMed: 23669137]
- Mann JJ, Emslie G, Baldessarini RJ, Beardslee W, Fawcett JA, Goodwin FK, Leon AC, Meltzer HY, Ryan ND, Shaffer D, Wagner KD. ACNP task force report on SSRIs and suicidal behavior in youth. *Neuropsychopharmacology*. 2006; 31:473–492. [PubMed: 16319919]
- Masi G, Liboni F, Brovedani P. Pharmacotherapy of major depressive disorder in adolescents. *Expert Opin Pharmacother*. 2010; 11:375–386. [PubMed: 20102303]
- McMillan KA, Asmundson GJG, Zvolensky MJ, Carleton RN. Startle response and anxiety sensitivity: subcortical indices of physiologic arousal and fear responding. *Emotion*. 2012; 12:1264–1272. [PubMed: 22775129]
- McNamara RK, Able JA, Rider T, Tso P, Jandacek R. Effect of chronic fluoxetine treatment on male and female rat erythrocyte and prefrontal cortex fatty acid composition. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010; 34:1317–1321. [PubMed: 20655971]
- Mikail HG, Dalla C, Kokras N, Kafetzopoulos V, Papadopoulou-Daifoti Z. Sertraline behavioural response associates closer and dose-dependently with cortical rather than hippocampal serotonergic activity in the rat forced swim stress. *Physiol Behav*. 2012; 107:201–206. [PubMed: 22771833]
- Miles L, Davis M, Walker D. Phasic and sustained fear are pharmacologically dissociable in rats. *Neuropsychopharmacology*. 2011; 36:1563–1574. [PubMed: 21471958]
- Mitchell JE, De Zwaan M, Roerig JL. Drug therapy for patients with eating disorders. *Curr Drug Targets CNS Neurol Disord*. 2003; 2:17–22. [PubMed: 12769809]
- Miura H, Kitagami T, Ozaki N. Suppressive effect of paroxetine, a selective serotonin uptake inhibitor, on tetrahydrobiopterin levels and dopamine as well as serotonin turnover in the mesoprefrontal system of mice. *Synapse*. 2007; 61:698–706. [PubMed: 17559097]
- Nagano M, Liu M, Inagaki H, Kawada T, Suzuki H. Early intervention with fluoxetine reverses abnormalities in the serotonergic system and behavior of rats exposed prenatally to dexamethasone. *Neuropharmacology*. 2012; 63:292–300. [PubMed: 22710353]
- National Research Council. Guidelines for the care and use mammals in neuroscience and behavioral research. Washington: National Academy Press; 2010.
- Nelson JC, Lu Pritchett Y, Martynov O, Yu JY, Mallinckrodt CH, Detke MJ. The safety and tolerability of duloxetine compared with paroxetine and placebo: a pooled analysis of 4 clinical trials. *Prim Care Companion J Clin Psychiatry*. 2006; 8:212–219. [PubMed: 16964316]
- Norcross M, Mathur P, Enoch AJ, Karlsson RM, Brigman JL, Cameron HA, Harvey-White J, Holmes A. Effects of adolescent fluoxetine treatment on fear-, anxiety- or stress-related behaviors in C57BL/6J or BALB/cJ mice. *Psychopharmacology (Berl)*. 2008; 200:413–424. [PubMed: 18594797]
- Oh JE, Zupan B, Gross S, Toth M. Paradoxical anxiogenic response of juvenile mice to fluoxetine. *Neuropsychopharmacology*. 2009; 34:2197–2207. [PubMed: 19440190]
- Parker G, Brotchie H. Gender differences in depression. *Int Rev Psychiatry*. 2010; 22:429–436. [PubMed: 21047157]

- Piccinelli M, Wilkinson G. Gender differences in depression: critical review. *Br J Psychiatry*. 2000; 177:486–492. [PubMed: 11102321]
- Pratt, LA.; Brody, DJ.; Gu, Q. Antidepressant use in persons aged 12 and over: United States, 2005–2008. NCHS data brief, no 76. Hyattsville, MD: National Center for Health Statistics; 2011.
- Reinecke MA, Curry JF, March JS. Findings from the treatment for adolescents with depression study (TADS): What have we learned? What do we need to know? *J Clin Child Adolesc Psychol*. 2009; 38:761–767. [PubMed: 20183660]
- Richards D. Prevalence and clinical course of depression: a review. *Clin Psychol Rev*. 2011; 31:1117–1125. [PubMed: 21820991]
- Sareen J. Anxiety disorders and risk for suicide: why such controversy? *Depress Anxiety*. 2011; 28:941–945. [PubMed: 22076969]
- Serretti A, Mandelli L. Antidepressants and body weight: a comprehensive review and meta-analysis. *J Clin Psychiatry*. 2010; 71:1259–1272. [PubMed: 21062615]
- Sher Y, Lolak S, Maldonado JR. The impact of depression in heart disease. *Curr Psychiatry Rep*. 2010; 12:255–264. [PubMed: 20425289]
- Shishkina GT, Kalinina TS, Dygalo NN. Effects of swim stress and fluoxetine on 5HT1A receptor gene expression and monoamine metabolism in the rat brain regions. *Cell Mol Neurobiol*. 2012; 32:787–794. [PubMed: 22453856]
- Sillaber I, Panhuysen M, Henniger MS, Ohl F, Kühne C, Pütz B, Pohl T, Deussing JM, Paez-Pereda M, Holsboer F. Profiling of behavioral changes and hippocampal gene expression in mice chronically treated with the SSRI paroxetine. *Psychopharmacology (Berl)*. 2008; 200:557–572. [PubMed: 18629477]
- Silva RC, Brandão ML. Acute and chronic effects of gepirone and fluoxetine in rats tested in the elevated plus-maze: an ethological analysis. *Pharmacol Biochem Behav*. 2000; 65:209–216. [PubMed: 10672971]
- Strekalova T, Couch Y, Kholod N, Boyks M, Malin D, Leprince P, Steinbusch HMW. Update in the methodology of the chronic stress paradigm: internal control matters. *Behav Brain Funct*. 2011; 7:9. [PubMed: 21524310]
- Substance Abuse Mental Health Services Administration. Results from the 2012 National Survey on Drug Use and Health: Mental Health Findings, NSDUH Series H-47, HHS Publication No. (SMA) 13–4805. Rockville, MD: Substance Abuse and Mental Health Services Administration; 2013.
- Tiihonen J, Lonngvisy J, Wahlbeck K, Klaukka T, Tanskanen A, Haukka J. Antidepressants and the risk of suicide, attempted suicide, and overall mortality in a nationwide cohort. *Arch Gen Psychiatry*. 2006; 63:1358–1367. [PubMed: 17146010]
- Thompson MR, Li KM, Clemens KJ, Gurtman CG, Hunt GE, Cornish JL, McGregor IS. Chronic fluoxetine treatment partly attenuates the long-term anxiety and depressive symptoms induced by MDMA ('ecstasy') in rats. *Neuropsychopharmacology*. 2004; 29:694–704. [PubMed: 14627999]
- Trouvin JH, Gardier AM, Chanut E, Pages N, Jacquot C. Time course of brain serotonin metabolism after cessation of long-term fluoxetine treatment in the rat. *Life Sci*. 1993; 52:187–192.
- Valluri S, Zito JM, Safer DJ, Zuckerman IH, Mullins CD, Korelitz JJ. Impact of the 2004 Food and Drug Administration pediatric suicidality warning on antidepressant and psychotherapy treatment for new-onset depression. *Med Care*. 2010; 48:947–954. [PubMed: 20856141]
- Velehorsch C, Pleau P, Vermani M, Furtado M, Klassen LJ. Understanding the role of adjunctive nonpharmacological therapies in management of the multiple pathways to depression. *Psychiatry Res*. 2014; 220(S1):S34–S44. [PubMed: 25539873]
- Vicente MA, Zangrossi H Jr. Involvement of 5-HT_{2C} and 5-HT_{1A} receptors of the basolateral nucleus of the amygdala in the anxiolytic effect of chronic antidepressant treatment. *Neuropharmacology*. 2014; 79:127–135. [PubMed: 24275045]
- Vorhees CV, Morford LR, Graham DL, Skelton MR, Williams MT. Effects of periadolescent fluoxetine and paroxetine on elevated plus-maze, acoustic startle, and swimming immobility in rats while on and off-drug. *Behav Brain Funct*. 2011; 7:41. [PubMed: 21974752]
- Wagner S, Müller C, Helmreich I, Huss M, Tadi A. A meta-analysis of cognitive functions in children and adolescents with major depressive disorder. *Eur Child Adolesc Psychiatry*. 2014; 24:5–19. [PubMed: 24869711]

Zorrilla EP. Multiparous species present problems (and possibilities) to developmentalists. Dev Psychobiol. 1997; 30:141–150. [PubMed: 9068968]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1.

Mean ($\pm$ SEM) body weights of male and female rats treated with vehicle, 2.5 or 10 mg/kg paroxetine (PAX), or 10 mg/kg fluoxetine (FLU) from PD 35 to PD 44. * Significant difference between vehicle- and fluoxetine-treated rats of the same sex. † Significant difference between vehicle- and 10 mg/kg paroxetine-treated rats of the same sex.

Fig. 2.

Mean ($\pm$ SEM) percent time spent in the open arms of the elevated plus maze. Male and female rats were treated with vehicle, 2.5 or 10 mg/kg paroxetine (PAX), or 10 mg/kg fluoxetine (FLU) from PD 35 to PD 44. * Significant difference between vehicle- and 10 mg/kg paroxetine-treated rats.

Fig. 3.

Mean ($\pm$ SEM) body weights of male and female rats treated with vehicle, 2.5, 5 or 10 mg/kg paroxetine (PAX), or 5, 10 or 20 mg/kg fluoxetine (FLU) from PD 35 to PD 44.

*Significantly different from vehicle-treated rats of the same sex.

Fig. 4.

Mean ($\pm$ SEM) percent time spent in the open arms (top panels), total head dips (middle panels), and closed arm entries (bottom panels) on the elevated plus maze. Male and female rats were treated with vehicle, 2.5, 5 or 10 mg/kg paroxetine (PAX), or 5, 10 or 20 mg/kg fluoxetine (FLU) from PD 35 to PD 44. * Significant difference from vehicle-treated rats.

Fig. 5.

Mean ($\pm$ SEM) time spent in light compartment (top panels) and light/dark compartment transitions (bottom panels) during a 10 min test in the light/dark box. Male and female rats were treated with vehicle, 2.5, 5 or 10 mg/kg paroxetine (PAX), or 5, 10 or 20 mg/kg fluoxetine (FLU) from PD 35 to PD 44. * Significant difference from vehicle-treated rats.

Fig. 6.

Mean ($\pm$ SEM) startle magnitude (top panels) and startle habituation (bottom panels) on the test day. Male and female rats were treated with vehicle, 2.5, 5 or 10 mg/kg paroxetine (PAX), or 5, 10 or 20 mg/kg fluoxetine (FLU) from PD 35 to PD 44. *Significant difference from vehicle-treated rats. †Significant difference from vehicle-treated male rats. ‡Significant difference from same-sex vehicle-treated rats.

Table 1

Mean (+SEM) sucrose consumption (grams) and preference for male and female rats ($n=11-12$ per group) after acute treatment on PD 45. Rats were treated with vehicle from PD 35 to PD 43 and given a single injection of vehicle, 2.5 or 10 mg/kg paroxetine (PAX), or 10 mg/kg fluoxetine (FLU) on PD 44.

Pretreatment	Sucrose Consumption		Sucrose Preference	
	Males	Females	Males	Females
Vehicle	63.9 (+6.1)	56.5 (+9.0)*	96.9 (+0.4)	87.9 (7+.1)*
PAX (2.5 mg/kg)	70.5 (+7.4)	66.7 (+10.9)*	96.1 (+1.4)	94.6 (+1.6)*
PAX (10 mg/kg)	59.5 (+7.8)	32.9 (+7.4)*	95.7 (+1.0)	78.9 (+1.9)*
FLU (10 mg/kg)	42.9 (+6.8) [†]	29.8 (+7.1)*, [†]	86.1 (+8.1) [†]	73.2 (+1.7)*, [†]

* Significantly different from male rats.

[†] Significantly different from vehicle-treated rats.

Table 2

Mean (+SEM) sucrose preference and difference scores (i.e., change from baseline) for male and female rats ($n=11-12$ per group) on PD 45. Rats were treated with vehicle, 2.5 or 10 mg/kg paroxetine (PAX), or 10 mg/kg fluoxetine (FLU) from PD 35 to PD 44.

Pretreatment	Sucrose Preference		Difference Scores	
	Males	Females	Males	Females
Vehicle	76.3 (+8.8)	81.4 (+7.9)	-10.7 (+0.6)	1.6 (+7.6)
PAX (2.5 mg/kg)	89.4 (+4.1)	83.6 (+8.1)	2.0 (+4.9)	13.6 (+7.9)
PAX (10 mg/kg)	73.2 (+11.1)	82.9 (+6.5)	-2.9 (+4.9)	-3.3 (+1.9)
FLU (10 mg/kg)	72.5 (+11.1)	76.3 (+11.9)	0.8 (+8.6)	4.2 (+3.3)

Note: Negative difference scores denote a decreased preference for sucrose relative to baseline, while positive scores denote an increased preference.

Table 3

Mean hippocampal monoamine content ($\pm$ SEM) of male and female rats ($n=6-7$ per group) on PD 45. Rats were treated with vehicle, 2.5 or 10 mg/kg paroxetine (PAX), or 10 mg/kg fluoxetine (FLU) from PD 35 to PD 44.

	5-HT	5-HIAA	5-HIAA/5-HT	NE
Males				
Vehicle	0.300 (± 0.06)	0.240 (± 0.01)	0.936 (± 0.14)	0.288 (± 0.02)
PAX (2.5 mg/kg)	0.274 (± 0.02)	0.268 (± 0.02)	0.981 (± 0.05)	0.344 (± 0.02)
PAX (10 mg/kg)	0.216 (± 0.03) [*]	0.224 (± 0.02)	1.099 (± 0.04)	0.336 (± 0.02)
FLU (10 mg/kg)	0.235 (± 0.02)	0.136 (± 0.01) [*]	0.691 (± 0.06) [*]	0.308 (± 0.03)
Females				
Vehicle	0.298 (± 0.02)	0.273 (± 0.02)	0.959 (± 0.12)	0.299 (± 0.02)
PAX (2.5 mg/kg)	0.248 (± 0.02)	0.276 (± 0.02)	1.119 (± 0.05)	0.288 (± 0.02)
PAX (10 mg/kg)	0.191 (± 0.03) [*]	0.138 (± 0.02) [†]	0.739 (± 0.04)	0.327 (± 0.03)
FLU (10 mg/kg)	0.271 (± 0.02)	0.237 (± 0.02) [*]	0.696 (± 0.07) [*]	0.376 (± 0.01)

Data are expressed as ng/mg wet weight tissue.

^{*} Significantly different from vehicle-treated rats.

[†] Significantly different from vehicle-treated rats of the same sex.

Table 4

Mean prefrontal cortex monoamine content ($\pm$ SEM) of male and female rats ($n=6-7$) on PD 45. Rats were treated with vehicle, 2.5 or 10 mg/kg paroxetine (PAX), or 10 mg/kg fluoxetine (FLU) from PD 35 to PD 44.

	5-HT	5-HIAA	5-HIAA/5-HT	NE
Males				
Vehicle	0.164 ($\pm$ 0.01)	0.114 ($\pm$ 0.01)	0.696 ($\pm$ 0.03)	0.072 ($\pm$ 0.005)
PAX (2.5 mg/kg)	0.156 ($\pm$ 0.01)	0.120 ($\pm$ 0.01)	0.778 ($\pm$ 0.07)	0.087 ($\pm$ 0.007)
PAX (10 mg/kg)	0.134($\pm$ 0.01)*	0.139 ($\pm$ 0.02)	1.027 ($\pm$ 0.17)	0.097 ($\pm$ 0.008)
FLU (10 mg/kg)	0.136 ($\pm$ 0.01)*	0.077 ($\pm$ 0.02)*	0.552 ($\pm$ 0.11)*	0.107 ($\pm$ 0.020) [†]
Females				
Vehicle	0.159 ($\pm$ 0.01)	0.106 ($\pm$ 0.01)	0.681 ($\pm$ 0.09)	0.092 ($\pm$ 0.004) [‡]
PAX (2.5 mg/kg)	0.154 ($\pm$ 0.01)	0.124 ($\pm$ 0.01)	0.798 ($\pm$ 0.05)	0.113 ($\pm$ 0.008)
PAX (10 mg/kg)	0.127 ($\pm$ 0.01)*	0.081 ($\pm$ 0.01)	0.641 ($\pm$ 0.07)	0.087 ($\pm$ 0.007)
FLU (10 mg/kg)	0.144 ($\pm$ 0.01)*	0.073 ($\pm$ 0.01)*	0.499 ($\pm$ 0.04)*	0.090 ($\pm$ 0.009)

Data are expressed as ng/mg wet weight tissue.

* Significantly different from vehicle-treated rats.

[†] Significantly different from vehicle-treated rats of the same sex.

Significantly difference from vehicle-treated male rats.

Table 5

Mean hippocampal monoamine content ($\pm$ SEM) of male and female rats ($n=7-8$ per group) after acute treatment on PD 45. Rats were treated with vehicle from PD 35 to PD 43 and then given a single injection of vehicle, 2.5 or 10 mg/kg paroxetine (PAX), or 10 mg/kg fluoxetine (FLU) on PD 44.

	5-HT	5-HIAA	5-HIAA/5-HT	NE
<i>Males</i>				
Vehicle	0.285 (+0.02)	0.253 (+0.03)	0.918 (+0.12)	0.354 (+0.02)
PAX (2.5 mg/kg)	0.282 (+0.03)	0.275 (+0.03)	0.967 (+0.07)	0.366 (+0.04)
PAX (10 mg/kg)	0.269 (+0.02)	0.224 (+0.02)	0.849 (+0.10)	0.361 (+0.04)
FLU (10 mg/kg)	0.320 (+0.03)	0.189 (+0.02)	0.635 (+0.08)*	0.398 (+0.03)
<i>Females</i>				
Vehicle	0.258 (+0.02)	0.257 (+0.03)	1.017 (+0.11)	0.322 (+0.02)
PAX (2.5 mg/kg)	0.271 (+0.03)	0.268 (+0.04)	0.979 (+0.08)	0.337 (+0.05)
PAX (10 mg/kg)	0.247 (+0.02)	0.247 (+0.04)	1.047 (+0.15)	0.335 (+0.04)
FLU (10 mg/kg)	0.314 (+0.03)	0.211 (+0.02)	0.668 (+0.04)*	0.386 (+0.03)

Data are expressed as ng/mg wet weight tissue.

* Significantly different from vehicle-treated rats.

Table 6

Mean prefrontal cortex monoamine content ($\pm$ SEM) of male and female rats ($n=7-8$) after acute treatment on PD 45. Rats were treated with vehicle from PD 35 to PD 43 and then given a single injection of vehicle, 2.5 or 10 mg/kg paroxetine (PAX), or 10 mg/kg fluoxetine (FLU) on PD 44.

	5-HT	5-HIAA	5-HIAA/5-HT	NE
<i>Males</i>				
Vehicle	0.161 (+0.02)	0.093 (+0.01)	0.633 (+0.07)	0.088 (+0.004)
PAX (2.5 mg/kg)	0.176 (+0.02)	0.120 (+0.01)	0.691 (+0.07)	0.100 (+0.008)
PAX (10 mg/kg)	0.156 (+0.02)	0.086 (+0.01)	0.570 (+0.06)	0.097 (+0.007)
FLU (10 mg/kg)	0.168 (+0.02)	0.085 (+0.01)	0.545 (+0.06)	0.091 (+0.003)
<i>Females</i>				
Vehicle	0.147 (+0.02)	0.108 (+0.01)	0.753 (+0.11)	0.091 (+0.008)
PAX (2.5 mg/kg)	0.148 (+0.03)	0.122 (+0.02)	0.744 (+0.13)	0.095 (+0.008)
PAX (10 mg/kg)	0.159 (+0.02)	0.097 (+0.01)	0.633 (+0.07)	0.112 (+0.004)
FLU (10 mg/kg)	0.194 (+0.02)	0.100 (+0.01)	0.539 (+0.05)	0.099 (+0.005)

Data are expressed as ng/mg wet weight tissue.