

# Repeated Fluoxetine Administration During Adolescence Stimulates Aggressive Behavior and Alters Serotonin and Vasopressin Neural Development in Hamsters

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Fluoxetine is the only selective serotonin reuptake inhibitor registered for the treatment of major depressive disorder in pediatric populations, despite reports that it is disproportionately associated with an array of adverse side effects that include agitation, hostility, and overt acts of pathological aggression and violence in youth. This study examined the effects of repeated adolescent fluoxetine administration on offensive aggression and the development of the serotonin (5HT) and vasopressin (AVP) neural systems modulating this behavior using pubertal Syrian hamsters (*Mesocricetus auratus*) as an adolescent-animal model. Adolescent hamsters administered fluoxetine were tested for offensive aggression using the resident/intruder test, sacrificed the following day, and, using immunohistochemistry, examined for 5HT and AVP afferent innervation/development to areas of the brain implicated in aggression control. Repeated exposure to a low dose (0.3 mg/kg/day) of fluoxetine during adolescence increased nearly all measures of offensive aggression (i.e., upright offensive attacks, lateral attacks, flank/rump bites, pursuits, flank marks), whereas measures of social interest (i.e., olfactory investigation, contact time), comfort (i.e., grooming), and locomotion (i.e., contact time, cage climbing) remained constant. Fluoxetine exposure also increased 5HT and AVP afferent development to brain areas implicated in aggressive behavior, most notably the latero-anterior hypothalamus (LAH)—an area of convergence for developmental and neuroplastic changes correlated with offensive aggression in hamsters. These data indicate that repeated administration of clinically relevant doses of fluoxetine during adolescent development directly stimulates aggressive behavior and alters LAH 5HT and AVP development, yet only alterations in AVP afferent development within the LAH correlate with the fluoxetine-induced aggressive behavioral phenotype.

*Keywords:* fluoxetine, adolescence, aggression, vasopressin, serotonin

Selective serotonin reuptake inhibitors (SSRIs) have been among the most widely prescribed medications in psychiatry for over a decade. Although there is a wealth of information regarding their effectiveness and safety in adults, limited data exists regarding whether they are safe for children. This notwithstanding, in 2006, the SSRI fluoxetine (Prozac) was the first drug approved by the United States Food and Drug Administration (FDA) for major depressive disorder (MDD) in children and adolescents (Bhatia & Bhatia, 2007; Safer, 2006), and to date, remains the only SSRI registered for treatment of MDD in the pediatric population. Since its approval, SSRI prescriptions have been on the rise in youth (and adult) populations (Birmaher, 1998; Coyle, 2000; Coyle et al., 2003; Kratochvil et al., 2006; Zito et al., 2002; Zito et al., 2006),

with over 24 million prescriptions for fluoxetine alone reported in the United States in 2010, representing a 7% increase from the prior year (SDI, 2011). This trend is alarming given that fluoxetine has been shown to be associated with an increased frequency and rate of an array of adverse side effect that include suicidal thoughts and actions, feelings of hostility, agitation, irritability, and overt acts of pathological aggression and violence (Cheung, Dewa, & Levitt, 2005; Edwards, Inman, Wilton, Pearce, & Kubota, 1997; Fava et al., 1991; Hammad, Laughren, & Racoosin, 2006; Inman, Kubota, Pearce, & Wilton, 1993; TADS, 2004; Moore, Glenmullen, & Furberg). Of particular concern is the increased incidence of hostility and aggression observed in children and adolescents treated with SSRIs, since the temporal onset of these adverse effects often occur within weeks of starting SSRI treatment (Cheung et al., 2005; Kratochvil et al., 2006), and, it is important to note, at low “starting” doses, that is, at the initial dose of 10–20 mg/day fluoxetine (Ahn, Yakutis, & Frazier, 2011; Cheung et al., 2005). Given the increase in the number of prescriptions for SSRIs like fluoxetine to youth, and the incidence of aggression reported in youth administered starting doses of fluoxetine, it is important to know whether exposure to low, clinically relevant doses of fluoxetine during periods of youth development, such as adolescence, directly predisposes users to increased aggressive behavior, and if so, how. Yet, it is surprising that relatively little, if any, preclinical research has focused on elucidating the effects of early

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fluoxetine exposure on aggressive behavior. In fact to date, it is unknown whether repeated, early fluoxetine administration has any effects on aggression and/or its underlying neurobiological correlates.

For over a decade, we have used pubertal male Syrian hamsters (*Mesocricetus auratus*) as an adolescent-animal model to investigate the effects of early drug exposure on the neurobiology of aggression (DeLeon, Grimes, Connor, & Melloni, 2002; Harrison, Connor, Nowak, & Melloni, 2000; Jackson et al., 2005; Knyshevski, Connor, Harrison, Ricci, & Melloni, 2005; Knyshevski, Ricci, McCann, & Melloni, 2005; Ricci, Connor, Morrison, & Melloni, 2007; Ricci, Grimes, & Melloni, 2004; see Melloni & Ricci, 2010 for a review). These studies identified the serotonin (5HT) and arginine vasopressin (AVP) neural systems in the anterior hypothalamus (AH) as points of convergence for developmental and neuroplastic changes that correlate with the aggressive phenotype. In hamsters, the AH exists at the center of a neural network of reciprocal connections between the bed nucleus of the stria terminalis (BNST), lateral septum (LS), medial amygdala (MeA) and ventrolateral hypothalamus (VLH), which regulate offensive aggression (Delville, De Vries, & Ferris, 2000). The activity of the entire network is regulated (at least partially) by the development and activity of AH 5HT and AVP. In this instance, AH 5HT activity inhibits offensive aggression that is normally facilitated by AH AVP (Ferris et al., 1997; Ferris, Stolberg, & Delville, 1999). The source of 5HT afferents to the AH are neurons located in the raphe nucleus (Delville et al., 2000), whereas the source of afferents releasing AVP into the AH are magnocellular AVP neurons located within the medial supraoptic nucleus (mSON), as well as neurons located in a small cluster centered directly within the AH proper in the nucleus circularis (NC) (Ferris, Axelson, Martin, & Roberge, 1989; Ferris, Gold, De Vries, & Potegal, 1990; Mahoney, Koh, Irvin, & Ferris, 1990). Recently we have shown that adolescent hamsters stimulated to respond aggressively following exposure to several drugs of abuse display increases in AVP levels, afferent innervation, and/or release in a ventro-lateral subregion of the AH, designated the latero-anterior hypothalamus (LAH; DeLeon et al., 2002; Grimes & Melloni, 2005; Harrison, Connor, Nowak, Nash, & Melloni, 2000; Jackson et al., 2005; Ricci, Rasakham, Grimes, & Melloni, 2006). This increase in LAH-AVP activity was commensurate with significant deficits in 5HT innervation/activity when compared with nonaggressive, vehicle-treated controls, together suggesting that increased LAH AVP and decreased 5HT development underlie adolescent, drug-induced offensive aggression (DeLeon et al., 2002; Grimes & Melloni, 2005; Harrison, Connor, Nowak, Nash et al., 2000; Jackson et al., 2005; Ricci et al., 2006); a notion supported by behavioral pharmacology studies employing AVP receptor antagonists and 5HT receptor agonists (DeLeon et al., 2002; Grimes & Melloni, 2005, 2002; Harrison, Connor, Nowak, Nash et al., 2000; Jackson et al., 2005; Ricci et al., 2006). During adolescent development, increases in LAH AVP development correlate with the temporal onset of the aggressive phenotype in drug-treated animals, supporting the view that at times of increased LAH AVP, animals respond more aggressively than when levels of AVP are low (Grimes, Ricci, & Melloni, 2007). However, reductions in AH 5HT development were observed well before the appearance of the aggressive phenotype, suggesting that 5HT may not play as large a role in the modulation of aggression as AVP does (Grimes et al.,

2007). Similarly, during withdrawal from adolescent drug exposure, reductions in LAH AVP innervation correlated with the return to the nonaggressive phenotype, whereas deficits in AH 5HT afferents existed throughout withdrawal, further implying that 5HT may not play as large a role in the modulation of aggression as AVP (Grimes & Melloni, 2006). Together, these data indicated that adolescent drug exposure had significant effects on the relationship between offensive aggression and LAH AVP, correlating alterations in LAH AVP with the aggressive phenotype. These data strengthened the idea that the interactions between adolescent drug exposure and LAH AVP might directly underlie drug-induced offensive aggression, while calling into question the role of AH 5HT and this response. It is possible that the increased incidence of hostility and aggression observed in youth administered SSRIs (e.g., fluoxetine) is also the result of alterations in AVP and/or 5HT neural development. To date, however, it is unknown whether adolescent fluoxetine exposure predisposes an animal to behave aggressively, and/or whether any behavioral effects of adolescent exposure correlate with an altered development of the AVP and/or 5HT neural systems.

In this study, we used pubertal hamsters as an adolescent model to examine the relationship between repeated early fluoxetine exposure, offensive aggression, and 5HT and AVP neural development. First, to investigate the hypothesis that repeated administration of clinically relevant doses of fluoxetine during adolescent development directly stimulated aggressive behavior in young animals, fluoxetine-treated hamsters were tested during late adolescence for offensive aggression using the resident/intruder test. Then, to investigate the hypothesis that increases in aggressive behavior in adolescent fluoxetine-treated animals would correlate with the altered development of neural systems implicated in aggression control, immunohistochemistry was utilized to visualize and quantify 5HT and AVP afferent fibers and varicosities within select brain regions in fluoxetine-treated hamsters.

## Method

### Adolescent-animal model

Adolescence in humans begins after puberty and ends near adulthood with sexual maturity, social awareness, and independence. A comparable "adolescent" period exists in pubertal male hamsters. Hamsters wean around postnatal Day 25 (P25), leave the nest (Dieterlen, 1959; Schoenfeld & Leonard, 1985), and interact with conspecifics to establish social relationships by P35 (Whitsett, 1975). Puberty occurs between P35 and P65, with a minimum breeding age of P42 (Festing; Miller, Whitsett, Vandenberg, & Colby, 1977; Vomachka, Ruppert, Clemens, & Greenwald, 1981). During the adolescent period then, hamsters attain sexual maturity, social responsiveness, and independence. The adolescent hamster is also well-suited for studying the effects of developmental fluoxetine exposure on offensive aggression (i.e., the form of aggression critical for establishing adult social relationships (Floody & Pfaff, 1977) because this form of aggression develops during adolescence (Dieterlen, 1959; Schoenfeld & Leonard, 1985) and is well-characterized in this species (Drickamer & Vandenberg, 1973; Floody & Pfaff, 1977; Lerwill & Makings, 1971; Pellis & Pellis, 1988a, 1988b).

For the experimental treatment paradigm, intact pubertal male hamsters (P21) were obtained from Charles River Laboratories (Wilmington, MA), individually housed in Plexiglas cages, and maintained at ambient room temperature on a reverse light:dark cycle of 14L:10D; lights on at 19:00. Food and water were provided ad libitum. For aggression testing, stimulus (intruder) males of equal size and weight to the experimental animals were obtained from Charles River one week prior to the behavioral test, group-housed at five animals per cage in large polycarbonate cages, and maintained as above to acclimate to the animal facility. To control for behavioral differences between stimulus animals, one day prior to the aggression test on experimental animals, all intruders were evaluated and prescreened for inherently low aggression (i.e., disengage and evade) and submission (i.e., tail-up freeze, flee, and fly-away) by placing intruder pairs in a neutral arena for 10 sec as described in a number of our previous studies (Delville, Melloni, & Ferris, 1998; Ferris et al., 1997; Ricci et al., 2004; Ricci, Knyshevski, I., & Melloni, 2005). The few stimulus hamsters (~8%) that displayed significantly low aggression or submissive postures were excluded from use in the behavioral assay. These animals were identified as individuals fleeing immediately to avoid interacting with a conspecific when tested in a neutral environment. Noninherently submissive animals initiated contact with conspecifics through olfactory investigations during the prescreening process. All experimental treatments and behavioral tests described below were administered during the first four hours of the dark cycle under dim-red illumination to control for circadian influences. All procedures were preapproved by the Northeastern University Institutional Animal Care and Use Committee (NU-IACUC).

## Experimental Treatment

In a series of four separate studies, adolescent (P27) hamsters received daily intraperitoneal injections of either fluoxetine or a saline vehicle for 30 consecutive days during early-to-late adolescence (P27–P56). On the first day (P27), animals were assigned to one of four groups ( $n = 10$ –25 animals per group), each receiving one of three doses of fluoxetine (0.3 mg/kg, 0.7 mg/kg, 1.0 mg/kg) or saline as control. The treatment regimen was designed to approximate the dose and duration of fluoxetine administered to children and adolescents, representing an average initial “starting” dose (15 mg/day, i.e., 0.3 mg/kg/day), a moderate dose (35 mg/day, i.e., 0.7 mg/kg/day), or a high dose (50 mg/day, i.e., 1.0 mg/kg/day), based on adolescent growth, development, and Tanner stage (Stang & Story, 2005; Tanner, 1962, 1969). The day following the last injection (P57), animals underwent behavioral testing.

## Behavioral Testing

**Offensive aggression.** In the first study, animals from fluoxetine ( $n = 10$ –20/dose) and vehicle ( $n = 10$ ) groups were tested for offensive aggression using the resident/intruder test, a well-characterized and ethologically valid model of offensive aggression in hamsters (Floody & Pfaff, 1977; Lerwill & Makings, 1971). Briefly, an intruder animal of similar size and weight was introduced into the home cage of the experimental animal (resident), and the resident was scored for: (a) general measures of offensive aggression (i.e., number of attacks and bites, latency to

attack and bite toward intruders) as previously described in Harrison, Connor, Nowak, & Melloni, 2000, and (b) more specific and targeted aggressive responses, including upright offensive postures, lateral attacks, aggressive pursuits, and flank/rump bites as described by Grimes, Ricci, & Melloni, 2003, to provide a more detailed account of the aggressive encounter between drug-treated residents and intruders. An attack was scored each time the resident animal would pursue and then either: (a) lunge toward and/or (b) confine the intruder by upright and sideways threat; each generally followed by a direct attempt to bite the intruder’s dorsal rump and/or flank target area(s). The latency to attack and bite was defined as the period of time between the beginning of the behavioral test and the first attack and bite of the residents toward an intruder. In the case of no attacks and/or bites, latencies were assigned the maximum time of the test duration (i.e., 600 s). Each aggression test lasted for 10 min and was videotaped and coded manually by two observers who were unaware of the hamsters’ experimental treatments. Differences in scores for all behaviors measured were less than 5% between the two observers. No intruder was used for more than one behavioral test. In a second study, fluoxetine- ( $n = 10$ /dose) and saline- ( $n = 10$ ) treated animals were scored for odor-induced flank marking, that is, a measure of social communication that is part of the larger ethogram of offensive aggression in hamsters (Floody & Pfaff, 1977; Lerwill & Makings, 1971), as described in Ferris, Axelson, Shinto, and Albers, 1987 and Johnston, 1975. Briefly, experimental animals were placed into a cage recently disseminated with the scent of a conspecific and scored for flank marking over a 5-min test period. Flank marks were recorded each time the hamster arched its back and vigorously rubbed its flank gland against the side or wall of its cage while moving in a forward direction. Flank-marking behavior was videotaped and measured by two independent observers uninformed of the treatment conditions.

**Behavioral activation.** In a third study, fluoxetine- ( $n = 10$ –20/dose) and saline- ( $n = 15$ ) treated animals were tested for an array of social, comfort, and motor behaviors to control for nonspecific activation effects of fluoxetine. Social interest was assessed by measuring physical contact time and social investigation. Contact time was defined as the period and duration of time during which the saline- or fluoxetine-treated resident initiated and maintained contact with the intruder, and social investigation was defined as the number of times the saline- or fluoxetine-treated resident would pursue and initiate an olfactory investigation of the intruder. Self-grooming was used as a measure of comfort behavior. For locomotion studies, a clean Plexiglas cage (22 cm  $\times$  28 cm open field) was subdivided into a four panel 11 cm  $\times$  14 cm grid and used to score open-field matrix crossings (i.e., line crosses) and escape attempts (i.e., wall climbing) of saline- and fluoxetine-treated hamsters.

## Immunohistochemistry

In a fourth study, aggressive, low-dose (0.3 mg/kg/day) fluoxetine-treated animals and nonaggressive, saline-treated controls ( $n = 12$ /group) were housed overnight as above. The day following the behavioral test for aggression (P58), fluoxetine- and saline-treated hamsters were anesthetized with Ketamine/Xylazine (80 mg/12 mg) and transcardially perfused with a 21 °C saline rinse, followed by a fixative solution of 4% paraformaldehyde.

Brains from animals were removed, postfixed for 90 min in the perfusion fixative, and cryoprotected in 30% sucrose-infused distilled water at 4° C overnight. Brains from fluoxetine and saline animals were then cut, using a freezing microtome, into two consecutive series of 35- $\mu$ m coronal sections and used for 5HT or AVP ( $n = 6$ /group/treatment) immunohistochemistry, as described previously (Grimes & Melloni, 2002; Grimes & Melloni, 2006; Grimes et al., 2007; Harrison, Connor, Nowak, Nash et al., 2000).

**5HT immunohistochemistry.** Free-floating sections from the brains of fluoxetine and saline animals were washed three times for 15 min ( $3 \times 15$  min) in phosphate-buffered saline (PBS) with 0.6% Triton X (Tx), pretreated with 3% H<sub>2</sub>O<sub>2</sub> in distilled water for 10 min, rinsed thoroughly with 0.6% PBSTx, then incubated in an antibody buffer containing 20% normal goat serum with 0.6% Tx-100 (Sigma Chemical Co., St. Louis, MO). Sections were then incubated in primary antiserum (1:1,000) for 5HT antirabbit (Protos Biotech, New York, NY), prepared in an antibody buffer for 24 hrs at 37° C. After primary incubation, sections were rinsed  $3 \times 15$  min with 0.6% PBSTx, incubated for 90 min in biotinylated, secondary goat antirabbit Immunoglobulin G (IgG; Vector Laboratories, Burlingame, CA) in 0.6% PBSTx, then rinsed again  $3 \times 15$  min with 0.6% PBSTx and incubated for 90 min in an avidin-biotin-complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) in 0.6% PBSTx. The peroxidase reaction was revealed as above, and the sections were mounted on gel-coated slides, air-dried, dehydrated through a series of alcohols, cleared with xylene, and coverslipped with Cytoseal (Stephens Scientific, Kalamazoo, MI).

**AVP immunohistochemistry.** Free-floating sections from the brains of fluoxetine and saline animals were washed three times for 15 min ( $3 \times 15$  min) in 0.1-M PBSTx, pretreated with 4.5% H<sub>2</sub>O<sub>2</sub> in distilled water for 10 minutes, rinsed thoroughly with 0.6% PBSTx, then treated with 1.5% sodium borohydride, and rinsed  $3 \times 15$  min in 0.6% PBSTx. Sections were incubated in antibody buffer containing 10% normal goat serum and 1% bovine serum albumin in 0.6% PBSTx for 90 min. Primary antibody (AVP, polyclonal, Chemicon; CA) was prepared in an antibody buffer diluted to a final concentration of 1:10,000, and incubation with free-floating sections was carried out overnight at 21° C on a rotation wheel. Sections were then rinsed  $3 \times 15$  min with 0.6% PBSTx, incubated for 90 min in biotinylated secondary goat antirabbit IgG (Vector Laboratories, Burlingame, CA.) in 0.6% PBSTx, then rinsed again  $3 \times 15$  minutes with 0.6% PBSTx and incubated for 90 min in an avidin-biotin-complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA.) in 0.6% PBSTx. The peroxidase reaction was revealed using 0.5% 3,3'-diaminobenzidine in distilled water as per manufacturer's recommendations (DAB Kit, Vectastain; Vector Laboratories, Burlingame CA.). The sections were mounted on gel-coated slides, air-dried, dehydrated through a series of alcohols, cleared with xylene and coverslipped with Cytoseal (Stephens Scientific, Kalamazoo, MI).

### Image Analysis

For immunohistochemistry, the density of 5HT and AVP immunoreactive fibers and varicosities (5HT- and AVP- ir) was determined within specific brain areas using the BIOQUANT NOVA 5.5 (R&M Biometrics, Nashville, TN) computer-assisted

microscopic-image-analysis software package as previously described (Grimes & Melloni, 2002; Grimes, Ricci, & Melloni, 2006; Grimes et al., 2007). The areas analyzed were selected based on previous data implicating these regions as part of the neural circuit important for aggressive behavior (Delville et al., 2000; Ferris et al., 1997; Melloni & Ricci, 2010). These areas included the AH proper, the LAH, the LH, the BNST, the LS, the MeA and central amygdala (CeA), and the VLH. Slides from each animal were coded by an experimenter unaware of the experimental treatment and BIOQUANT NOVA 5.8 image-analysis software running on a Pentium III CSI open PC (R&M Biometrics, Nashville, TN.) was utilized to identify the brain regions of interest at low power (4X) using the dark field setting on a Nikon E600 microscope. At this magnification, a standard computer-generated box was drawn to fit within the particular region of interest (ROI). Then, each brain region was assigned a separate and distinct ROI parcel at under 20X magnification; 5HT-ir and AVP-ir labeling was then calibrated with the ROI as a particular wavelength using a standard RGB scale. Quantification of 5HT-ir and AVP-ir elements was performed manually by the experimenter using the BIOQUANT software. Three to six independent measurements were taken from several consecutive sections of each animal ( $n = 12$ ) per treatment group. The number of 5HT-ir and AVP-ir elements was determined for each ROI and standardized per  $100 \times 100 \mu$ m parcel for regional comparison purposes. The number of 5HT-ir and AVP-ir elements was then averaged for each brain region per hamster and used for statistical analysis.

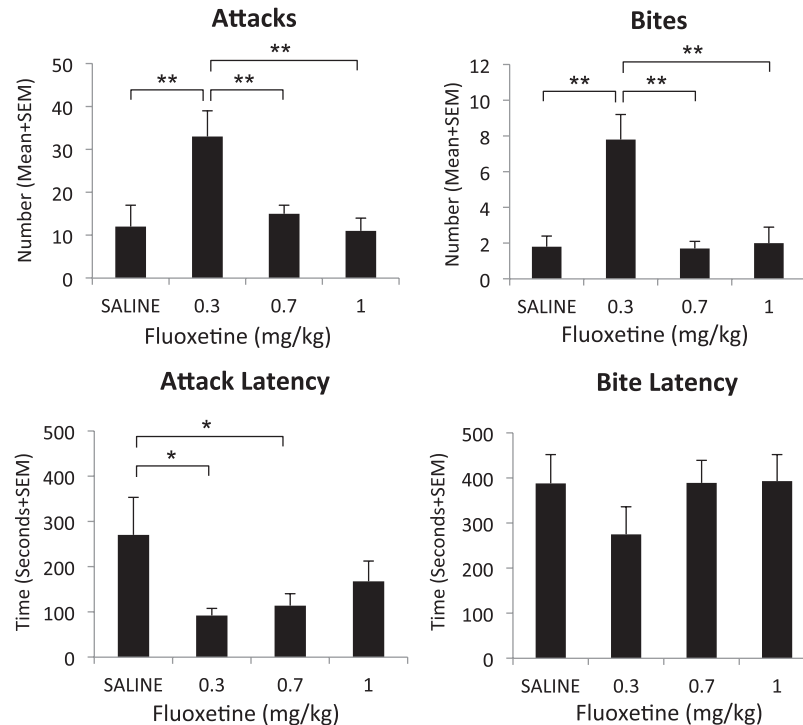
### Statistics

Results from aggression tests were compared across dose conditions. Behavioral data were compared using ANOVA followed by Fischer's Protected Least Significant Difference post hoc (two-tailed) tests. Immunohistochemical data from each area examined were analyzed between vehicle and fluoxetine groups using Student's *t* tests. Only animals that successfully completed the 10-min resident/intruder test period and fell within two standard deviations from the mean for all respective groups were included in the final behavioral data analyses. Similarly, only brains that were accurately prepared for immunohistochemistry were included in the neurobiological analysis. The  $\alpha$  level for all experiments was set at 0.05.

## Results

### Behavioral Effects of Adolescent Fluoxetine Administration

**Fluoxetine effects on offensive aggression.** Fluoxetine administration produced an overall effect on aggression intensity, that is, number of attacks,  $F_{(3,43)} = 5.88$ , and bites,  $F_{(3,43)} = 5.86$ ;  $p < .01$  each, with significant aggression-eliciting effects observed only at the low dose (i.e., 0.3 mg/kg) of fluoxetine (see Figure 1). At this dose, fluoxetine-treated animals directed significantly more attacks,  $t(17) = 3.17$  and bites,  $t(17) = 3.26$  onto intruders ( $p < .01$  each) than did saline controls. Low (0.3 mg/kg) dose fluoxetine animals also directed significantly more attacks toward intruders than did moderate (0.7 mg/kg) dose,  $t(22) = 3.15$  or high (1.0 mg/kg) dose,  $t(22) = 3.94$  animals ( $p < .01$  each). Similarly,



**Figure 1.** Effects of fluoxetine administration during adolescence on general measures of offensive aggression. Low-dose fluoxetine (0.3 mg/kg/day) exposure significantly increased the total number of attacks and bites of fluoxetine-treated residents onto intruders compared with saline-treated control animals. Low-dose (0.3 mg/kg/day) fluoxetine-treated residents also directed significantly more attacks and bites toward intruders than did animals administered moderate- (0.7 mg/kg/day) or high- (1.0 mg/kg/day) dose fluoxetine during adolescence. Low-dose fluoxetine (0.3 mg/kg/day) exposure also significantly decreased the latency to the first attack of fluoxetine-treated residents onto intruders compared with saline-treated control animals, or animals administered moderate- (0.7 mg/kg/day) dose fluoxetine during adolescence. In contrast, fluoxetine administration during adolescence at any dose failed to significantly affect the fluoxetine-treated residents' latency to bite intruders. Bars denote SEM; \*  $p < 0.05$ . \*\*  $p < 0.01$ .

Low-dose fluoxetine animals directed significantly more bites onto intruders than moderate-,  $t(22) = 3.68$  or high-,  $t(22) = 3.53$  dose animals ( $p < .01$  each). Fluoxetine exposure also produced an overall effect on aggression initiation, that is, latency to first attack,  $F_{(3,43)} = 2.94$ ,  $p < .05$ , but not first bite,  $F_{(3,43)} = 1.11$ ,  $p > .05$  (see Figure 1). Here, animals treated with low- and moderate-dose fluoxetine were significantly quicker to attack than saline controls,  $t(17) = 2.56$  and  $t(21) = 3.36$ ,  $p < .05$  each.

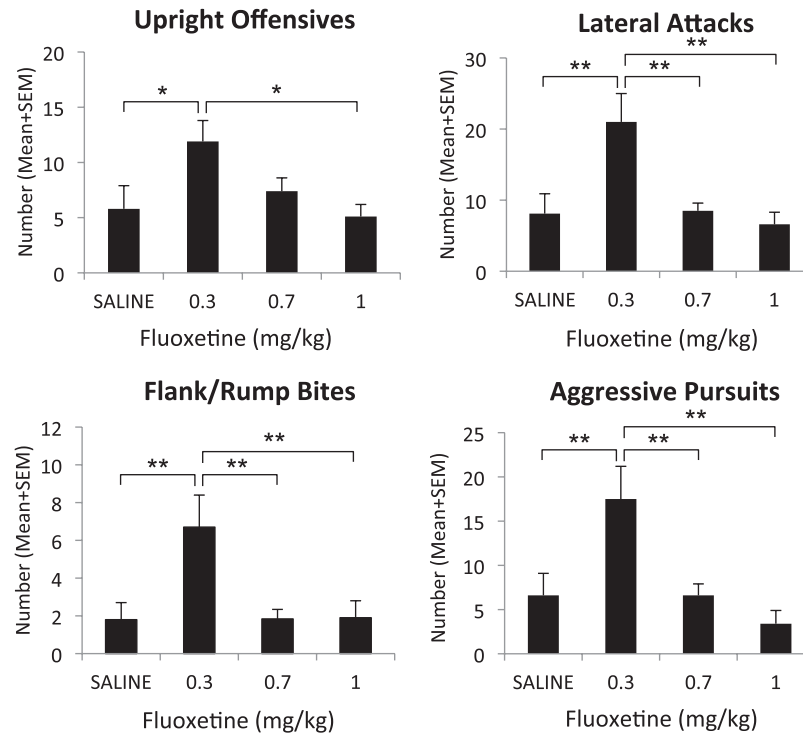
Fluoxetine exposure produced an overall effect on targeted offensive responses, that is, number of upright offensive attacks,  $F_{(3,43)} = 2.85$ ,  $p < .05$ , lateral attacks,  $F_{(3,43)} = 6.59$ ,  $p < .001$ , flank/rump bites,  $F_{(3,43)} = 6.47$ ,  $p < .01$ , and aggressive pursuits,  $F_{(3,43)} = 6.64$ ,  $p < .001$ , again with the significant effects observed only at low doses (0.3 mg/kg) of fluoxetine (see Figure 2). Here, upright offensive attacks,  $t(17) = 2.10$ ,  $p < .05$ , lateral attacks,  $t(17) = 3.30$ ,  $p < .01$ , flank/rump bites,  $t(17) = 3.46$ ,  $p < .01$ , and aggressive pursuits,  $t(17) = 2.73$ ,  $p < .01$  were significantly increased compared with saline controls. Also, low-dose animals directed significantly more lateral attacks, flank/rump bites, and aggressive pursuits than did moderate-dose,  $t(22) = 3.57$ ,  $t(22) = 3.55$ ,  $t(22) = 3.39$ , or high-dose,  $t(22) = 4.10$ ,  $t(22) = 3.96$ ,  $t(22) = 4.32$ , animals ( $p < .01$  each). Low-dose

fluoxetine animals also directed significantly more upright offensive attacks than those administered high doses,  $t(22) = 2.67$ ,  $p < .05$ .

Fluoxetine exposure also produced an overall effect on odor-induced flank marking,  $F_{(3,48)} = 11.83$ ,  $p < .001$ , with a significant effect observed again only at low doses (0.3 mg/kg) of fluoxetine (see Figure 3). Here, animals directed significantly more flank marks than saline controls,  $t(18) = 4.54$ ,  $p < .001$ . Low-dose fluoxetine animals also directed significantly more flank marks than did moderate-dose animals,  $t(18) = 3.44$ , or high-dose animals,  $t(17) = 3.45$  ( $p < .01$  each). Similarly low-dose fluoxetine enhanced the initiation of flank marking, as fluoxetine animals were quicker to flank mark than saline controls,  $t(18) = 2.42$ ,  $p < .05$  (see Figure 3).

**Fluoxetine effects on behavioral activation.** Fluoxetine exposure at all doses produced no significant activation effects on any measure of social, comfort, or motor behavior, that is, contact time,  $F_{(3,46)} = 0.71$ ; olfactory investigation,  $F_{(3,32)} = 0.56$ ; grooming,  $F_{(3,32)} = 0.63$ ; line crosses,  $F_{(3,28)} = 0.48$ ; or wall climbing,  $F_{(3,28)} = 0.57$  (see Table 1).

**Physiological effects of adolescent fluoxetine administration.** Given the significant effects of repeated, low-dose (0.3 mg/kg)



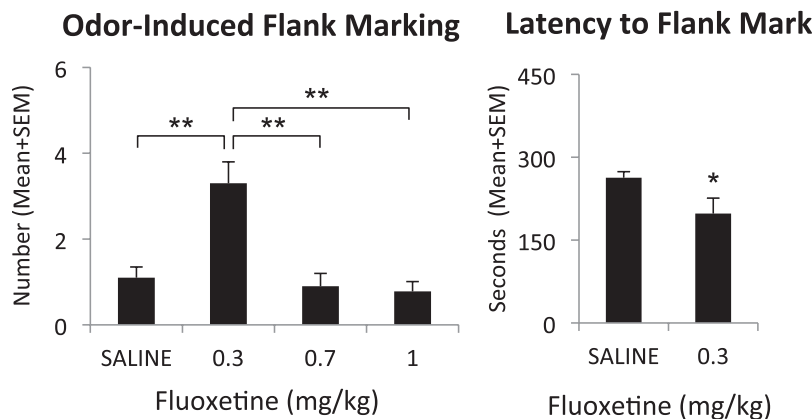
**Figure 2.** Effects of fluoxetine administration during adolescence on targeted measures of offensive aggression. Low-dose fluoxetine (0.3 mg/kg/day) exposure significantly increased the total number of upright offensive and lateral attacks, flank/rump bites, and aggressive pursuits of fluoxetine-treated residents onto intruders, compared with saline-treated control animals. Low-dose (0.3 mg/kg/day) fluoxetine-treated residents also directed significantly more upright offensive attacks toward intruders than did animals administered high- (1.0 mg/kg/day) dose fluoxetine during adolescence, and directed significantly more lateral attacks onto intruders than did animals administered either moderate- (0.7 mg/kg/day) or high- (1.0 mg/kg/day) dose fluoxetine during adolescence. Low-dose (0.3 mg/kg/day) fluoxetine-treated residents also directed significantly more flank/rump bites and aggressive pursuits toward intruders than did animals administered moderate- (0.7 mg/kg/day) or high- (1.0 mg/kg/day) dose fluoxetine during adolescence. Bars denote *SEM*; \*  $p < 0.05$ . \*\*  $p < 0.01$ .

fluoxetine administration on nearly all measures of offensive aggression, this dose was selected for studies examining fluoxetine effects on 5HT/AVP neural development and body weight.

**Fluoxetine effects on the 5HT/AVP neural systems.** Aggressive, fluoxetine-treated animals displayed alterations in the density of 5HT- and AVP-containing afferent fibers and varicosities in brain regions implicated in aggression control (see Figure 4). For 5HT, as we have described previously (Grimes & Melloni, 2002; Grimes & Melloni, 2006; Grimes et al., 2007), in nonaggressive, saline-treated hamsters, a high density of 5HT-containing afferent fibers and varicosities were found in the LAH, BNST, CeA, LS, MeA, and VLH (Figure 4 inset depicts the LAH). By comparison, aggressive, fluoxetine-treated hamsters showed significant increases in the density of 5HT-ir afferent fibers and varicosities within the LAH,  $t(10) = 3.01$ ; BNST,  $t(10) = 2.81$ ; LS,  $t(10) = 2.32$ ; MeA,  $t(10) = 2.99$ ; and VLH,  $t(10) = 3.34$  ( $p < .01$  each), but not the CeA,  $t(10) = 0.61$ ,  $p > .05$ . For AVP, as we observed previously (Jackson et al., 2005), nonaggressive, saline-treated animals possessed a high density of AVP-ir in neurons in the mSON and within afferents from these neurons projecting into the LAH (Melloni & Ricci, 2010), whereas a less dense pattern of AVP-ir was observed in neurons in the NC and afferents projecting

into the AH proper (Figure 4 inset). Likewise, in nonaggressive, saline-treated control animals a moderate density of AVP-ir was observed in neurons in the ISON and within afferents projecting laterally into the LH. By comparison, aggressive, fluoxetine-treated hamsters showed a significant increase in the density of AVP-ir afferent fibers than did nonaggressive, saline controls, but only within a precise (and important) subregion of the hypothalamus. Specifically, fluoxetine-treated hamsters possessed a significant increase in the density of AVP-ir afferent fibers within the LAH brain region,  $t(10) = 2.24$ ,  $p < .05$ . No significant differences were observed in the density of AVP-ir afferents within the AH proper,  $t(10) = 0.04$ ,  $p = .965$ , nor the LH,  $t(10) = 0.18$ ,  $p = .856$  in aggressive, fluoxetine-treated hamsters, compared with nonaggressive, saline controls.

**Fluoxetine effects on body weight.** The repeated administration of low doses of (0.3 mg/kg) fluoxetine during adolescent development had no significant overall effects on body weight gain over the treatment period, compared with saline-treated (control) littermates (see Figure 5). On the first day of treatment, at P27, initial body weights were not significantly different ( $\pm$  *SEM*) between fluoxetine- (63.15  $\pm$  4.1 g) and vehicle- (64.08  $\pm$  4.9 g) treated hamsters. Following 28 days of treatment with fluoxetine,



**Figure 3.** Effects of fluoxetine administration during adolescence on communicative measures of offensive aggression. Low-dose fluoxetine (0.3 mg/kg/day) exposure significantly increased the total number of flank marks displayed by fluoxetine-treated residents compared to saline-treated control animals. Low dose (0.3 mg/kg/day) fluoxetine-treated residents also displayed significantly more flank marks than did animals administered moderate- (0.7 mg/kg/day) or high- (1.0 mg/kg/day) dose fluoxetine during adolescence. Low-dose fluoxetine (0.3 mg/kg/day) exposure also significantly decreased the latency to the first flank mark of fluoxetine-treated residents compared to saline-treated control animals, Bars denote SEM; \*  $p < 0.05$ . \*\*  $p < 0.01$ .

animals showed a significant increase in body weight ( $116.54 \pm 7.7$  g), as did the vehicle-treated control group ( $122.09 \pm 10.5$  g). However, there was no significant difference in total body weight gain observed between vehicle- ( $58.01 \pm 5.6$  g) and fluoxetine- ( $53.39 \pm 3.7$  g) treatment groups,  $t(18) = 0.18$ ,  $p > .05$ .

### Discussion

The SSRIs are among the most widely prescribed medications for the treatment of MDD in youth, despite reports they are associated with an increased incidence of a number of serious adverse side effects, including suicidality, aggression, and violence, as well as worsening depression and anxiety in this population (Ahn et al., 2011; Cheung et al., 2005; Hammad et al., 2006; Kratochvil et al., 2006; TADS, 2004). Recently, a number of preclinical studies have emerged examining the effects of developmental fluoxetine exposure on a series of behaviors ranging from anxiety, fear, and behavioral despair to stress-induced behavioral reactivity and sexual motivation. For instance, adolescent mice and rats repeatedly administered fluoxetine at doses approx-

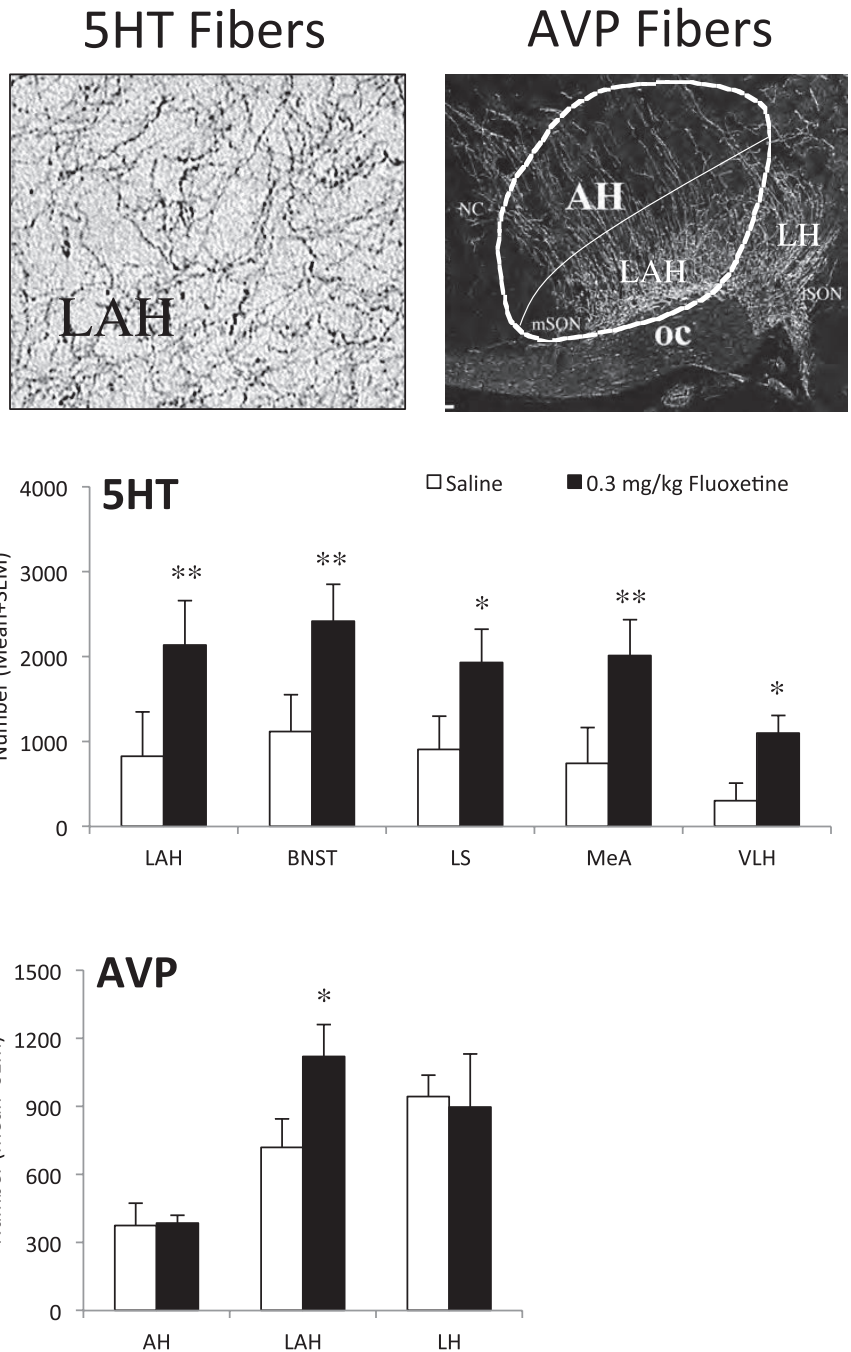
imating those administered clinically to youth show increased anxiety-like behavior (Iñiguez, Warren, & Bolanos-Guzman, 2010; Oh, Zupan, Gross, & Toth, 2009), decreased- (Iñiguez et al.) or increased- (Bhansali, Dunning, Singer, David, & Schmauss, 2007) behavioral despair, and disrupted normal sexual behavioral responses (Iñiguez et al.), but other studies have shown no changes in performance on behavioral assays of fear, anxiety, and/or stress (Burghardt, Sullivan, McEwen, Gorman, & LeDoux, 2004; LaRoche & Morgan, 2007; Norcross et al., 2008). Interestingly, many of the differences reported in anxiety-like behavior disappear when animals are tested following a fluoxetine washout period (Oh et al., 2009), suggesting that the adverse effects of developmental fluoxetine exposure may be transient. In contrast, fluoxetine exposure during earlier developmental periods, for example, during the neonatal period or early in the postnatal period, increased anxiety- and depression- like behaviors that lasted into adulthood in rodents (Ansorge, Morelli, & Gingrich, 2008; Ansorge, Zhou, Lira, Hen, & Gingrich, 2004; Maciag et al., 2006; Popa, Lena, Alexandre, & Adrien, 2008), indicating that fluoxetine exposure at early stages

Table 1

*Effects of Fluoxetine Administration During Adolescence on General Measures of Behavioral Activation (n = 9–16/group)*

Behavior	Treatment Regimen (mg/kg)			
	Saline	0.3 Fluoxetine	0.7 Fluoxetine	1.0 Fluoxetine
Social behavior ( $\pm SD$ )				
Contact time (s)	$335.7 \pm 81$	$358 \pm 66.7$	$315 \pm 76.6$	$323 \pm 81.4$
Olfactory investigation	$9.6 \pm 4.3$	$10.8 \pm 3.9$	$9.4 \pm 4.2$	$9.7 \pm 3.2$
Comfort behavior ( $\pm SD$ )				
Self grooming	$2.7 \pm 1.4$	$2.3 \pm 1.1$	$2.6 \pm 0.6$	$2.5 \pm 1.2$
Locomotion behavior ( $\pm SD$ )				
Line crosses	$31.4 \pm 8.9$	$35.7 \pm 9.4$	$37.3 \pm 9.9$	$30.6 \pm 10.8$
Wall climbing	$5.4 \pm 3.2$	$4.3 \pm 2.6$	$4.9 \pm 4.1$	$5.1 \pm 3.6$

*Note.* Fluoxetine administration during adolescence at any dose failed to significantly affect measures of social (i.e., contact time and olfactory investigation), comfort (i.e., grooming), or motor (i.e., line crosses and wall climbing) behaviors.  $n = 10$ –20/group.



*Figure 4.* Effects of low-dose (0.3 mg/kg) fluoxetine administration during adolescence on 5HT and AVP neural development in brain areas implicated in aggression control. Fluoxetine exposure significantly increased the total number of 5HT and AVP afferent fibers compared with saline-treated control animals. Fluoxetine-treated animals possessed significantly more 5HT afferent fibers (as depicted in the inset—40X magnification) in the latero-anterior hypothalamus (LAH), bed nucleus of the stria terminalis (BNST), lateral septum (LS), medial amygdala (MeA), and ventrolateral hypothalamus (VLH) than did their saline-treated counterparts. Fluoxetine-treated animals also displayed significantly more AVP afferent fibers in select regions of the hypothalamus (as depicted in the inset—10X magnification). Specifically, animals administered fluoxetine during adolescent development possessed significantly more AVP afferent fibers in the LAH subdivision of the AH. No significant differences in afferent fibers were observed in the anterior hypothalamus proper (AH) nor the lateral hypothalamus (LH) between fluoxetine- and saline- treatment groups. Bars denote S.E.M. \*  $p < 0.05$ . \*\*  $p < 0.01$ . ISON = lateral supraoptic nucleus. mSON = medial supraoptic nucleus. NC = nucleus circularis, oc = optic chiasm.

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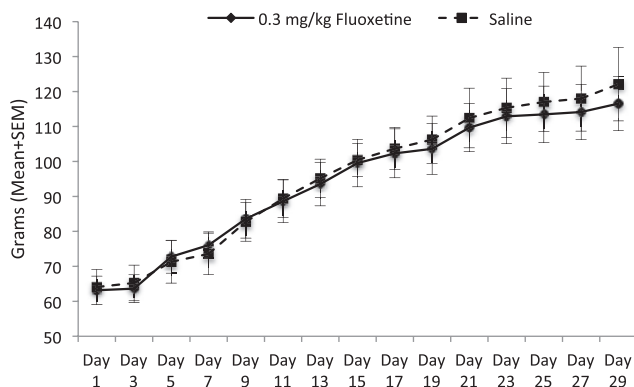


Figure 5. Effects of low-dose (0.3 mg/kg) fluoxetine administration during adolescence on body weight. Fluoxetine administration failed to significantly affect the total amount of weight gained over the adolescent treatment period. Bars denote SEM.

of development may produce lasting behavioral abnormalities, and thus present lifelong challenges to the individual. These findings indicate that when administered during development, fluoxetine can both increase and decrease anxiety- and depression-like responses, exhibiting dose, treatment regimen, and strain-specific effects. It is interesting that, given the association of fluoxetine with pathological aggression and violence in youth, it is surprising that of all these studies investigating a link between fluoxetine and its adverse side effects in developing animal models, none have examined the effects of repeated developmental fluoxetine administration on aggressive behavior.

A number of preclinical studies have, however, examined the influence of acute fluoxetine exposure on aggression, showing that acute fluoxetine administration suppresses aggressive behavior in adult mice (Pinna, 2010; Pinna, Costa, & Guidotti, 2005), rats (Homborg, Schiepers, Schoffemeer, Cuppen, & Vanderschuren, 2007), and fish (Lynn, Egar, Walker, Sperry, & Ramenofsky, 2007). Similarly, in adult hamsters, acute fluoxetine administration inhibits high levels of aggression normally expressed in adult animals repeatedly exposed to smaller conspecifics (i.e., trained fighters; Ferris et al., 1997) and those administered testosterone (Delville, Mansour, & Ferris, 1996). Similarly, across several adolescent hamster models, acute fluoxetine suppresses aggression displayed in animals repeatedly exposed to drugs of abuse, namely anabolic steroids (Grimes & Melloni, 2002) or cocaine (DeLeon et al., 2002). In contrast, other acute studies have shown that fluoxetine facilitates aggression in both adult and developmental animals. For instance, in Gulf toadfish (*Opsanus beta*), acute fluoxetine increased the number of aggressive acts made by dominant individuals onto subordinates (McDonald, Gonzalez, & Sloman, 2011), but in hamsters, the acute administration of fluoxetine produced differential responses in dose-dependent adolescent and adult males (Taravosh-Lahn, Bastida, & Delville, 2006). In this latter study, acute fluoxetine, at all doses, inhibited agonistic behavior in adult hamsters, whereas in adolescent hamsters, higher doses (i.e., 20 mg/kg) reduced offensive responses and lower doses (i.e., 10 mg/kg) enhanced them. Although these acute studies provide important information, they are limited in scope to the immediate pharmacologic effect (i.e., transiently increased 5HT

neural signaling) of high-dose (> 10 mg/kg) fluoxetine on aggressive behavior. Given that a repeated, substantially lower dose treatment schedule of fluoxetine (0.3–1.0 mg/kg/day) is the norm clinically, and that such a regimen typically has delayed behavioral effects, a more clinically applicable experimental strategy would be to examine the effects of repeated, lower dose fluoxetine administration on aggressive behavior following a substantial period of exposure. Unfortunately, few studies have adopted such a strategy, and none to date have examined the effect of repeated fluoxetine administration on aggressive behavior in developing animal models.

In the current study, adolescent hamsters who were repeatedly administered a low dose of fluoxetine (i.e., 0.3 mg/kg/day) for 28 days, displayed a highly escalated aggressive phenotype. It is interesting to note, this level of aggression met or exceeded that of trained fighters (Ferris et al., 1997; Schwartz, Ricci, & Melloni, 2012), suggesting that aggression may be maximized in adolescent animals administered fluoxetine. Importantly, fluoxetine-treated adolescents targeted their offensive responses, that is, a hallmark feature of mature, adult aggressive behavior (Taravosh-Lahn & Delville, 2004). Yet, here, adolescent hamsters were tested for aggression in the absence of prior social experience, therefore, fluoxetine exposure alone was sufficient to generate adolescent animals with a mature, highly escalated offensive aggressive phenotype. This finding was similar to that observed in adolescent hamsters, where a single exposure to fluoxetine precipitated the maturation of attack targets by residents (Taravosh-Lahn et al., 2006). These data differ however, in that adolescent animals in this acute-exposure study were administered a significantly higher dose of fluoxetine (i.e., 10 mg/kg), and tested for offensive responses during peak exposure times (i.e., 2 hr following administration). Therefore, circulating levels of the drug and its corresponding pharmacological influences were likely to be high, as fluoxetine has a half-life of approximately 8–15 hr (including its metabolites) in rodents (Raap & Van De Kar, 1999). In the current study, adolescent hamsters repeatedly exposed to low, clinically relevant doses of fluoxetine were tested for aggression > 24 hr following the final administration, a time frame at which no significant pharmacologic effects on aggression have been observed (Carrillo, Ricci, Coppersmith, & Melloni, 2009), essentially negating any pharmacologic influence of circulating levels of fluoxetine and/or its metabolites on aggression. It is possible that exposure to low doses of fluoxetine for a repeated time period during adolescent development stimulates aggression not by a direct pharmacological effect on 5HT reuptake, but rather by slowly altering the activity and/or development of neural circuits that control aggressive behavior. This possibility would support clinical data that associate a repeated low-dose administration of a fluoxetine regimen with an increased incidence of hostility and aggression in children and adolescents (Ahn et al., 2011; Cheung et al., 2005; Kratochvil et al., 2006).

In hamsters, the AH lies at the center of a network of reciprocal connections, regulates offensive aggression (Delville et al., 2000), and the activity of this entire network is modulated by the development/activity of the 5HT and AVP neural systems (Ferris et al., 1997; Ferris et al., 1999). Using several adolescent-animal models, we found that adolescent exposure to a number of drugs of abuse, facilitates a highly escalated aggressive phenotype, which is correlated with marked alterations in 5HT development/activity

within this neural network (DeLeon et al., 2002; Grimes & Melloni, 2002; Grimes et al., 2007; Harrison, Connor, Nowak, Nash et al., 2000; Jackson et al., 2005). For instance, adolescent hamsters administered low (but not moderate or high) doses of cocaine expressed a highly escalated aggressive phenotype that was modulated by AH 5HT activity (DeLeon et al., 2002; Harrison, Connor, Nowak, & Melloni, 2000; Knyshevski, Ricci et al., 2005; Ricci et al., 2007; Ricci et al., 2004). Behavioral data from these studies reveal a strikingly similar response to adolescent animals administered fluoxetine in the current study, suggesting a similar neurobehavioral and/or neurodevelopmental mechanism of action. From a neurobiological standpoint, brains of cocaine-treated hamsters had fewer 5HT afferents within the AH, as well as the VLH, MeA and CeA (DeLeon et al., 2002), indicating that cocaine suppressed the development of the aggression-suppressing 5HT system. Given that cocaine and fluoxetine share similar pharmacologic properties (i.e., both have high affinity for 5HT transporters), and that adolescent animals exposed to low doses of the two drugs express similar, highly aggressive behavioral profiles, we hypothesized that adolescent exposure to fluoxetine would also inhibit 5HT neural development within one or more of the brain sites important for aggression control in hamsters, facilitating the generation of the highly aggressive phenotype. Contrary to this hypothesis, we found that adolescent fluoxetine exposure enhanced 5HT development to nearly all subcortical brain sites implicated in the control of aggression. This finding is consistent with data from previous studies indicating that fluoxetine exposure increases the density of serotonin afferent innervation (i.e., the density and branching of 5HT-containing afferent fibers) in various brainstem, limbic, and cortical areas in adult rats (Zhou, Huang, Kecojevic, Welsh, & Koliatsos, 2006). These data, together with findings from the current study, support the idea that fluoxetine-induced aggression may be modulated by an increase in 5HT afferent development to subcortical brain areas implicated in aggression control. It is possible that by enhancing 5HT development, adolescent fluoxetine exposure increases the extent to which 5HT neurons innervate synaptic partners, perhaps functionally activating neural circuits stimulating aggression. Indeed, although 5HT's action on aggression has been shown to be mostly inhibitory, stimulatory effects of 5HT on aggressive behavior have been observed acting through 5HT Type-3 receptors (Derkach, Surprenant, & North, 1989; Maricq, Peterson, Brake, Myers, & Julius, 1991; Rudissar, Pruus, Skrebuhhova, Allikmets, & Matto, 1999; Sugita, Shen, & North, 1992). Recently, we localized 5HT3 receptors to many of the brain sites implicated in aggression control in hamsters, including the AH (Carrillo, Ricci, Schwartz, & Melloni; Ricci et al., 2004), and found that 5HT3 receptor stimulation enhanced aggression in adolescent hamsters (Ricci et al., 2004; Ricci, Knyshevski, I., Melloni Jr., R.H., 2005), concomitant with an increase in 5HT3 receptors and activity in the AH (Ricci et al., 2004). Thus, it is plausible that neurons located in the AH brain region, that is, most notably AH AVP neurons, receive an enhanced *excitatory* 5HT input as a result of adolescent fluoxetine exposure, culminating in the stimulation of AH AVP and offensive aggression in these animals. Alternatively, we also localized 5HT1A heteroreceptors to many of the brain sites implicated in aggression control in hamsters, including the LAH (Melloni & Ricci, 2010; Ricci et al., 2006). In these studies and others, we found that 5HT1A-receptor stimulation blocked the high levels of

offensive aggression observed in hamsters chronically exposed to select drugs of abuse during adolescence (Knyshevski, Ricci et al., 2005; Ricci et al., 2006), and that aggressive adolescent hamsters had a decrease in 5HT1A receptors and activity in the AH (Ricci et al., 2006). Thus, it is possible that chronic exposure to fluoxetine during adolescent development down regulates (and/or desensitizes) 5HT1A-receptor activity in brain regions implicated in aggression control. There is evidence for this mechanism, as acute fluoxetine exposure has been shown to transiently reduce 5HT1A-receptor localization and receptor binding in rats and cats (Aznavour et al., 2006; Riad et al., 2004), at least in part due to internalization of the receptor (Riad et al., 2004). However, several studies have shown that chronic fluoxetine exposure has little or no effect on 5HT1A-receptor density and/or binding (Aznavour et al., 2006; Riad et al., 2008), suggesting that acute and chronic exposure may have differential effects on the development/activity of the 5HT neural system. These differences notwithstanding, further investigation of the role of select 5HT receptors in the adolescent fluoxetine-induced aggressive response is warranted, as is an investigation of the development and activity of select neural systems implicated in aggression control expressing 5HT receptors.

In accord with this notion, hypothalamic AVP neurons express various 5HT receptors (Melloni & Ricci, 2010), and previously, we found that hamsters stimulated to respond hyperaggressively following adolescent exposure to select drugs of abuse display marked alterations in AVP development/activity within the hypothalamus, particularly within the AH (DeLeon et al., 2002; Grimes & Melloni, 2002; Grimes et al., 2007; Harrison, Connor, Nowak, Nash et al., 2000; Jackson et al., 2005). For instance, as for 5HT, we showed that adolescent cocaine-induced offensive aggression was modulated by AH AVP activity and release (Jackson et al., 2005), indicating that cocaine enhanced the activity of the aggression-stimulating AVP system in the AH. Given the aforementioned similarities between cocaine, fluoxetine, and the highly aggressive behavioral phenotype that both of these drugs induced, we hypothesized that adolescent exposure to fluoxetine would enhance the AVP development within the hypothalamic brain site important for aggression control in hamsters, that is, the LAH. In accord with this hypothesis, we found that chronic exposure to low doses of fluoxetine during adolescence enhanced the development of the AVP neural system in a select subregion of the AH, that is, the LAH. This neuroanatomical finding is important, as this subdivision of the AH has been directly implicated in the facilitation of aggression in a number of our investigations examining the neurobehavioral effects of adolescent drug exposure on aggression in hamsters. For instance, enhanced AVP development and production in the LAH, and activation of LAH AVP release and signaling, were critical for the temporal onset and maintenance of the anabolic steroid-induced aggression in adolescent animals (Carrillo, Ricci, & Melloni, 2011; Grimes et al., 2006, 2007; Harrison, Connor, Nowak, Nash et al., 2000; Melloni & Ricci, 2010). Similarly, Although aggressive, adolescent cocaine-treated hamsters showed no differences in AH AVP development or production from controls, they did show significant increases in AVP release into the AH (Jackson et al., 2005). Further, the odor-induced flank-marking data presented here support the notion that adolescent fluoxetine exposure increases AVP activity within the AH. In hamsters, flank marking is highly dependent upon AVP development and activity within the AH (Ferris, Albers, We-

solowski, Goldman, & Luman, 1984; Ferris et al., 1996; Ferris, Irvin, Potegal, & Axelson, 1990). The central administration of exogenous AVP to the AH increases flank marking in hamsters that can be reversed by the application of AVP receptor blockers (Ferris et al., 1984; Ferris, Pollock, Albers, & Leeman, 1985). Together, these data suggest that fluoxetine stimulates aggression by increasing the development/activity of the LAH AVP neural system, supporting our earlier assertion that at times of increased AH/LAH AVP tone, animals respond with higher levels of aggression than when levels of AH/LAH AVP are low (Grimes & Melloni, 2006; Grimes et al., 2006, 2007). These data support the hypothesis that the interactions between adolescent fluoxetine exposure and the LAH AVP neural system might underlie fluoxetine-induced aggressive behavior. However, from a neuroanatomical standpoint, it is also particularly interesting that no significant differences in AVP afferent development were observed within the AH proper (i.e., AVP afferents within the dorsal aspects of the AH proper) or the LH (i.e., AVP afferents leading, for the most part, to the posterior pituitary; Mahoney, 1990) in response to chronic adolescent exposure to fluoxetine in these studies. Taken together, these data highlight the potential for a role of enhanced development and activity of a small, select population of centrally projecting AVP neurons into the LAH brain region in adolescent fluoxetine-induced offensive aggression.

In summary, these studies provide the first examination of the effects of repeated fluoxetine administration to adolescents on offensive aggression and the neural systems modulating this behavior. These findings show that repeated administration of low, clinically relevant doses of fluoxetine during adolescence increases aggression and alters the development of the 5HT and AVP systems in a fashion consistent with the aggressive phenotype. Although not all research on the neurobiology of aggression is in agreement, these findings suggest a link between repeated fluoxetine administration, AVP/5HT neural development, and the stimulation of aggression in adolescent animals.

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