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Prenatal Exposure to Selective Serotonin Reuptake Inhibitors (SSRI) Increases Aggression and Modulates Maternal Behavior in Offspring Mice

ABSTRACT: *Selective serotonin reuptake inhibitors (SSRI) are commonly prescribed antidepressant drugs in pregnant women. SSRIs cross the placental barrier and affect serotonergic neurotransmission in the fetus. Although no gross SSRI-related teratogenic effects were reported, infants born following prenatal exposure to SSRIs are at higher risk for various developmental abnormalities. The aim of this study was to examine the effects of prenatal SSRI on social and maternal behavior in mice. To this end, pregnant female dams were exposed to saline or fluoxetine (FLX) throughout pregnancy, and the behavior of the offspring was examined. The results indicate that in utero FLX increased aggression in adult males and delayed emergence of maternal behavior in adult females. Social exploration and recognition memory were not affected by prenatal FLX exposure. These findings support the notion that alterations in the development of serotonergic pathways following prenatal exposure to SSRIs are associated with changes in social and maternal behavior throughout life.* © *2015 Wiley Periodicals, Inc.* Dev Psychobiol 58:71–82, 2016.

Keywords: *serotonin-selective reuptake inhibitors (SSRIs); fluoxetine; social behavior; aggression; social exploration; social memory; object memory; maternal behavior; nest building; pup retrieval; ICR (CD1) mice*

INTRODUCTION

Maternal depression during the period surrounding childbirth is a common psychiatric disorder, estimated to affect 17% of women in industrial societies (Alwan, Reefhuis, Rasmussen, & Friedman, 2011; Kendall-Tackett & Hale, 2010; Weikum, Mayes, Grunau, Brain, & Oberlander, 2013). Maternal depression bears negative consequences on pregnancy and infant growth, development and long term mental health (Alwan et al., 2011; Goodman, 2007). Depression treatments include antidepressant medications, psychotherapy, social support, and exercise. In some cases, antidepressants are the best and the most effective course of treatment (Kendall-Tackett & Hale, 2010).

Women suffering from depression during pregnancy often use antidepressant medications. Epidemiological data showed that 2–3% of pregnant women use Selective Serotonin Reuptake Inhibitors (SSRI) antidepressants (Bakker, Kolling, van den Berg, de Walle, & de Jong van den Berg, 2008; Cooper, Willy, Pont, & Ray, 2007; Mitchell et al., 2011; Olivier, Blom, Arentsen, & Homberg, 2011). SSRI drugs readily cross the placental barrier and impact the development and function of fetal bodily systems (Hendrick et al., 2003; Olivier et al., 2011; Yonkers et al., 2009). Human

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studies indicated that prenatal exposure to SSRIs increased the risk for low birth weight and preterm birth (Chambers, Johnson, Dick, Felix, & Jones, 1996), and for persistent pulmonary hypertension (Chambers et al., 2006). Prenatal SSRI exposure was also associated with a self-limiting neonatal withdrawal syndrome, including tremor, restlessness and rigidity (Laine, Heikkinen, Ekblad, & Kero, 2003; Moses-Kolko et al., 2005; Olivier et al., 2011).

Behavioral studies suggested an association between prenatal SSRI exposure and changes in social and emotional behavior. For example, in 3 years old children prenatal SSRI exposure was associated with an increase in internalized behavior, including social withdrawal, anxiety and depressive symptoms, increased emotionality, somatic complains, and sleep problems (Oberlander et al., 2010). In addition, following in utero SSRI exposure umbilical cord blood drug levels were positively correlated with externalized behavior, including aggression and attention deficit/hyperactivity in 4 years old children (Oberlander et al., 2007). Reports further indicated changes in facial reaction to pain and a shorter recovery time from pain in toddlers exposed to SSRI in utero (Oberlander et al., 2005). Studies also described impaired gross motor, social–emotional and adaptive behaviors in infants prenatally exposed to SSRIs (Hanley, Brain, & Oberlander, 2013).

Animal studies support these findings demonstrating increased risk for low birth weight and cardiomyopathy following a prenatal SSRI exposure (Olivier et al., 2011; Vorhees et al., 1994). Behavioral studies in rodents following perinatal exposure to SSRIs showed an increase in behavioral despair, reduced play behavior, diminished novel object exploration, decreased conspecific interactions, and reduced pain sensitivity (Hansen, Sanchez, & Meier, 1997; Lee, 2009; Lisboa, Oliveira, Costa, Venancio, & Moreira, 2007; Rodriguez-Porcel et al., 2011). It should be noted, however, that the prenatal development in rodents is not equivalent to that of humans. Studies showed that rodent brains are less developed at birth compared to human babies (Dobbing & Sands, 1979; Romijn, Hofman, & Gramsbergen 1991), thus conclusions from animal studies should be drawn with caution.

In summary, the literature suggests that prenatal SSRI exposure alters various forms of social behaviors in human and rodents. However, a thorough assessment of the life-long changes of many aspects of this behavior is lacking. Specifically, the long-lasting effects of prenatal SSRI on aggression and maternal behavior were not reported. Moreover, many studies lack clear distinction between the effects of prenatal and neonatal effects of SSRI exposure. In addition, possible gender differences in the response to prenatal SSRI were overlooked in most studies (Schellinck, Cyr, & Brown, 2010). Hence, the following study aims to examine the long-lasting effects of prenatal SSRI exposure on multiple aspects of social behavior in male and female mice.

MATERIALS AND METHODS

Animals

Subjects were male and female offspring of ICR (CD1) mice purchased from Harlan Laboratories (Jerusalem, Israel). Subject were born and raised at the Academic College of Tel Aviv-Yaffo Animal facility. Room temperature was $22 \pm 2^{\circ}$C, with a reversed 12 hr light/dark cycle (lights on at 7:00 PM). Water and solid food pellets were provided ad libitum. Animal care procedures were approved by the National Israeli Committee of Animal Care and Use. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

Behavioral Tests

Novel Object Exploration. The goal of this test was to assess the level of exploratory behavior. To this end, investigation of a novel object was evaluated. Subjects were placed individually in an observation unit for 5 min to allow acclimation, after which one of two novel objects of different texture and color was introduced into the observation cage. The order by which objects were presented and the location of the objects within the observation unit were determined randomly. Total duration of exploration of the novel object was examined for 5 min. Exploration of an object included deliberate physical contact, sniffing, touching, and climbing over the object (Rodriguez-Porcel et al., 2011).

Object Memory Test. The goal of this test was to assess object recognition memory by the ability of mice to differentiate between familiar and novel objects (Bevins & Besheer, 2006). Object memory test was conducted 10 min after the termination of the *novel object exploration* test, during which animals remained in the observation cage. Two different objects were introduced at the same time at the two opposite corners of the unit: (1) Familiar: the same object that was used in the *novel object exploration* test was presented at the same corner as in the former test; (2) Unfamiliar: a novel object was presented at the opposite corner of the observation cage. The two objects differed in texture and color. Duration of investigation of the two objects was assessed for 5 min. Exploration of an object included deliberate physical contact, sniffing, touching, and climbing over the object (Bevins & Besheer, 2006).

Social Preference Test. The goal of this test was to assess the preference for a social stimulus over an inanimate object. This test was conducted 10 min after the termination of the *object memory test*, during which animals remained in the observation cage. Animals were presented with two stimuli:

(1) Familiar object—the novel object that was used for the *object memory test*, presented at the same side of the observation cage. (2) Animal: a same-sex 4 weeks old conspecific. Stimulus animals were allowed to freely roam in the observation cage; however, their behavior was not recorded. Duration of investigation of the object and the stimulus animal were assessed for 5 min (Rodriguez-Porcel et al., 2011).

Social Exploration and Social Memory. Subjects were placed individually in the observation unit for 5 min of acclimation. To assess *social exploration*, subjects were introduced with a same-sex 4 weeks old stimulus mouse for 5 min. Social exploration of the experimental subjects was recorded. At the end of the observation stimulus animals were removed from the observation cage and subjects remained in the cage for 10 min recess. Following recess, social recognition memory was assessed using the *social memory* test (Bevins & Besheer, 2006). Subjects were introduced with two stimulus mice placed at two opposite corners of the observation unit: (1) Familiar: the same stimulus mouse that was used in the previous trial; (2) Unfamiliar: a novel same-sex, 4-weeks old conspecific. Duration of social exploration and number and duration of aggressive attacks were recorded for 5 min (Crawley, 2007). In both tests, stimulus animals were allowed to freely roam in the observation unit. The behavior of the stimulus animals was not recorded and data were based on the behavior of the experimental subjects only. Social investigation of a stimulus animal was defined as intentional physical contact initiated by the experimental subject, including sniffing, following, and climbing over or under the juvenile conspecific. In cases were aggressive behavior was noted, the number of aggressive attacks, the total duration of attacks and the percentage of aggressors per group were also assessed. Aggressive attacks included mount, bite and chase with physical contact exhibited by the experimental subject towards the stimulus animals (Avitsur, Stark, Sheridan, 2001; Avitsur, Stark, Dhabhar, Kramer, & Sheridan, 2003).

Maternal Behavior. Female mice display a range of maternal behaviors, including nest building, gathering young into the nest, incubation, feeding, and protecting of the young. Female mice typically prepare a nest before parturition. Once the pups are born, nursing is the main part of maternal behavior, and pups are weaned through a gradual nonaggressive process after about 3 weeks (Crawley, 2007; Russell & Leng, 1998; Weber & Olsson, 2008). Accordingly, two tests were conducted in order to assess the quality of maternal behavior: the nest quality test during late pregnancy and the pup retrieval test following parturition.

Nest quality. Nest quality was examined once a Day on GD14-GD16. Female subjects were removed from the home-cage along with all nesting material. Fresh, pre-weighed nesting material was placed in the cage, and subjects were immediately returned into the cage. Nest quality was examined 4 hr later on a scale of 1–4 (1 = no nest and nesting material largely untouched; 2 = low quality flat nest; 3 = a recognizable nest with low walls; 4 = high quality nest, with walls higher than mouse body height on most of its circumference) (Seip & Morrell, 2008). In addition, length, height, width, weight, and the presence of a roof and a floor were recorded (Crawley, 2007).

Pup retrieval. Pregnant female subjects were examined twice a Day (morning and evening), from the 16 Day of pregnancy, for the presence of parturition. Litter size was noted on postnatal Day (PND) 1. Pup retrieval test was conducted once a Day on PND 2–4. Two pups were taken out of the nest and placed outside the nest on two opposite sides of the home cage. The time elapsed until the dam returned the pups back into the nest was noted. If the pups were not returned to the nest after 10 min, the pups were returned to the nest by the observer (Crawley, 2007).

Procedure

Male and female mice were housed together until pregnancy was determined by the presence of a vaginal plug (gestational Day (GD) 0). From GD1 throughout pregnancy, females were housed individually and injected daily with fluoxetine (FLX, 10 mg/kg/day, s.c. in a volume of 10 ml/kg, Santa Cruz Biotechnology, Inc., Dallas, TX), or with equal volumes of sterile saline (SAL) ($n = 10$ dams/group). The dose used in this study was chosen based on our earlier studies and several previous reports using similar doses (e.g., Avitsur, Levy, Goren, & Grinshpahet, 2015a; Bairy, Madhyastha, Ashok, Bairy, & Malini, 2007; da-Silva, Altenburg, Malheiros, Thomaz, & Lindsey, 1999; Forcelli & Heinrichs, 2007; Hodes, Hill-Smith, Suckow, Cooper, & Lucki, 2010). At birth, the number of pups per litter was noted. On PND1 litters were weighed and culled to 10 pups. Systematic observations of dams and pups, including assessment of nest building, pup retrieval to the nest and pup survival from PND1 until weaning indicated that maternal behavior was not altered by the prenatal treatment and all pups survived until weaning (data not shown). Thus, it is suggested that this procedure did not induce significant withdrawal symptoms.

Offspring were weaned at the age of 21–23 days and housed in same-sex cages with their littermates. Offspring prenatally exposed to SAL or FLX underwent behavioral testing twice, at the age of 28–31 days (young) and again at the age of 49–52 days (adult), except where otherwise specified. Subjects ($n = 40$) were sampled from their litters according to sex and prenatal treatment. Up to two animals from the same sex and litter were included in the same experimental group. The study included four groups ($n = 10$/group): (I) Males prenatally exposed to SAL; (II) Males prenatally exposed to FLX; (III) Females prenatally exposed to SAL; (IV) Females prenatally exposed to FLX.

All behavioral tests except for maternal behavior were conducted in a transparent Plexiglas observation unit (size 40×40 cm, height 35 cm). Experimental materials and the observation unit were cleaned with an ethanol solution (70%) between animals to ensure the absence of remaining olfactory cues between trials. Behavioral tests were performed during the first half of the dark phase of the diurnal cycle under red

light illumination. Tests were conducted according to the following order: *novel object exploration, object memory*, and *social preference* tests were conducted on the same experimental day. *Social exploration* and *social memory* tests were conducted on the same experimental day; 1–3 days following the novel object exploration, object memory and social preference tests. Animals were allowed 10 min recess between tests that were performed on the same day. During the 10 min recess, animals remained in the observation cage. Different stimulus animals were used for the social preference and the social memory tests. Behavioral observations were recorded and later coded using the Noldus Observer Video-Pro software package (Noldus Information Technology, Sterling, VA).

Following behavioral tests, SAL and FLX female offspring underwent a breeding procedure at the age of 61–62 days. Male ICR mice were introduced into the female's cages until pregnancy was determined by the presence of vaginal plug (GD0). Females were housed individually and were undisturbed throughout pregnancy except for behavioral examinations. Additionally, on PND1, litter size was noted. Maternal behavior of female offspring was examined using the nest quality and pup retrieval tests as described.

Statistical Analysis

Unless otherwise specified, outlier observations (deviating from the mean ± 2 standard deviations range) were excluded from the analysis. Following exclusions all groups included at least eight subjects. In the social exploration and social memory tests, the variability of social exploration was high, resulting in a large number of outliers. Further analysis revealed that the source of this variability is the increased aggression exhibited by some of the subjects, which did not have a normal distribution. Thus, most of the outliers were in fact subjects that showed aggressive behavior. As aggressive behavior was common and a part of the focus of this study, it was decided that outliers should not be excluded from the analysis of the social exploration and social memory tests.

The results are presented as means (M) and standard deviations (SD). For statistical analysis of variance of within and between groups, ANOVA with repeated measures test was applied with prenatal treatment (SAL/FLX) and sex (male/female) as the between subjects factors and age (young/adult) as the within subjects factor. Additional within subject factors are stated below where appropriate. For the comparison of groups with two within subject variables an ANOVA with repeated measures was used. For the comparison between two groups an independent T test was applied. For all post-hoc comparisons, other than the aggression tests, the Bonferroni Post-hoc test was used. For post-hoc comparisons of the aggression tests condition Simple Contrast were used on account of evidence from other studies about male rather than female aggression. For the nest quality test, to standardize the objective measurements (length, height, width, weight and the presence of a roof and a floor) a z-score was calculated for each of the different measurement and a mean z score was calculated for each separate Day of assessment. Differences were considered significant at $p < .05$ and marginally significant at $p < .1$.

RESULTS

Litter Size and Pup Weight

T-test revealed that prenatal treatment had no significant effect on litter size at birth and average pup weight on PND1 (Litter size: Sal: $M = 10.8$, SD $= 3.4$; FLX: $M = 11.3$, SD $= 2.8$, $p > .1$; Pup weight: Sal: $M = 1.8$, SD $= .2$; FLX: $M = 1.9$, SD $= .13$, $p > .1$).

Novel Object Exploration

ANOVA revealed a significant effect for age on the duration of object exploration ($F(1,36) = 8.67$, $p = .01$), indicating that adult mice investigated the object longer than young mice (Young: $M = 38.86$, SD $= 19.98$; Adult: $M = 53.23$, SD $= 21.94$ s). Prenatal treatment and sex had no significant effect on the duration of object exploration ($p = .49$ and .35, respectively).

Object Memory Test

To compare the duration of exploration of the familiar and the unfamiliar objects an ANOVA was performed with an additional within subject variable: object type (familiar/unfamiliar). ANOVA revealed a significant main effect for object type ($F(1,34) = 23.85$, $p = .001$), indicating that mice explored the unfamiliar object more than the familiar one. An interaction was also found between the effects of age and object type ($F(1,34) = 6.87$, $p = .01$), indicating that the preference for the unfamiliar object was higher in adult mice (Young-familiar object: $M = 38.69$, SD $= 24.43$; Young-unfamiliar object: $M = 49.10$, SD $= 30.51$; Adult-familiar object: $M = 32.11$, SD $= 13.10$; Adult-unfamiliar object: $M = 64.48$, SD $= 31.97$). There was no significant effect for prenatal treatment on object memory ($p > .7$). An additional ANOVA was performed on the proportion of time spent exploring the unfamiliar object (unfamiliar object exploration duration divided by total exploration duration) as the dependent variable, sex and treatment as between subjects factors and age as a within subject factor. No significant treatment effect or interactions were found ($p > .1$).

Social Preference Test

To compare the duration of exploration of the object and the stimulus mouse an ANOVA was performed with an additional within subject variable: target type (object/animal). ANOVA revealed an interaction between the effects of age and target type ($F(1,34) = 40.88$, $p = .001$), indicating that in adult mice the duration of exploration of the stimulus mouse was significantly higher compared to object exploration. Additionally, there was an interaction between age, sex,

and prenatal treatment on the duration of exploration ($F(1,34 = 4.87$, $p = .03$), indicating that in young females, FLX significantly increased duration of animal exploration compared to SAL ($p = .036$). FLX had no significant effect on social preference in all other groups (Fig. 1).

Social Exploration

ANOVA revealed a significant interaction between age and sex on the duration of social exploration ($F(1,36 = 4.44$, $p = .04$), indicating that in adult animals the duration of exploration of the stimulus mouse was higher in males compared to females. There was no significant effect for prenatal treatment on social exploration ($p > .1$, Table 1).

Aggression in the Social Exploration Test. Aggressive behavior was not exhibited in young animals and was at negligible levels in females (Wimer & Fuller, 1966).

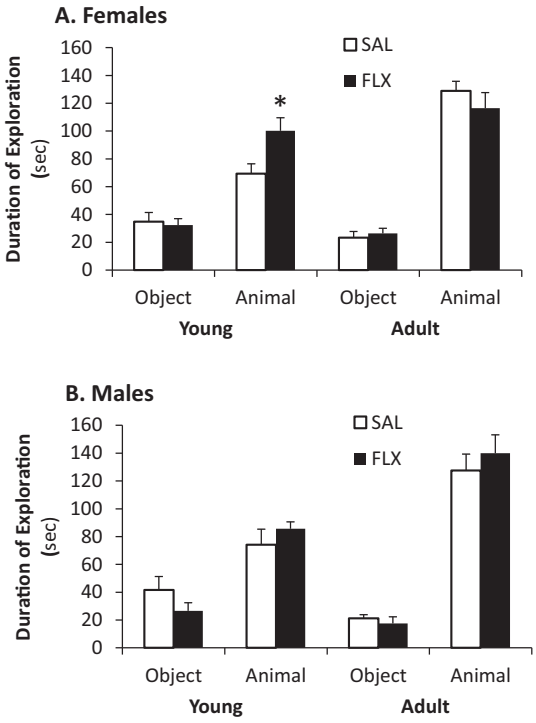

FIGURE 1 Effect of prenatal exposure to saline (SAL) or fluoxetine (FLX) on the duration of exploration of an object or a stimulus mouse in female (A) and male (B) subjects in the *Social Memory Test*. All animals presented a significant preference for the stimulus mouse over the object. In addition, the duration of investigation was higher in adult compared to young subjects. In young females, prenatal FLX treatment significantly increased exploration of the stimulus mice compared to SAL controls. Data are presented as mean ± SD. *Significantly different compared to the corresponding saline group.

Thus, the analysis of aggressive behavior included the adult males only. ANOVA with prenatal treatment as a between subjects factor revealed a significant main effect for prenatal treatment on the number of aggressive attacks ($F(1,18) = 4.58$, $p = .046$), and the duration of aggressive attacks ($F(1,18) = 4.58$, $p = .046$), indicating in adult males prenatal FLX significantly increased the number and duration of aggressive attacks compered to SAL controls (Fig. 2). Fisher's exact test was conducted to assess the effect of prenatal treatment on the proportion of male mice that engaged in aggressive behavior. This analysis revealed that there was no significant difference between SAL and FLX males in the proportion of aggressors (1 of 10 SAL males and 4 of 10 FLX males showed aggression, $p > .1$).

Social Memory

To compare the duration of exploration of the familiar and unfamiliar stimulus mice ANOVA was performed with an additional within subject variable: target type (familiar/unfamiliar). ANOVA revealed an interaction between age and sex on the duration of social exploration ($F(1,36 = 5.33$, $p = .03$), indicating that in young animals the duration of exploration was higher in females compared to males. There was no significant effect of prenatal treatment on the duration of the social exploration of the stimulus mice and no significant preference for the unfamiliar over a familiar target (Table 2). An additional ANOVA was performed on the proportion of time spent exploring the unfamiliar mouse (unfamiliar mouse exploration duration divided by total exploration duration) as the dependent variable, sex and treatment as between subjects factors and age as a within subject factor. No significant treatment effect or interactions were found ($p > .1$).

Aggression in the Social Memory Test. Similar to the social exploration test, aggression was not exhibited in young animals and was at negligible levels in females

Table 1. Effect of Prenatal Exposure to Saline (SAL) or Fluoxetine (FLX) on the Duration of Social Exploration of a Same-Sex 4-Weeks Old Conspecific in the *Social Exploration* Test

	Female		Male	
	Young	Adult	Young	Adult
SAL	94.7 (43.2)	95.1 (34.0)	84.1 (28.4)	106.9 (39.6)
FLX	91.4 (45.8)	99.2 (30.1)	69.0 (28.4)	107.4 (32.9)

Data are presented as mean (SD) in seconds. The duration of exploration of the stimulus mouse was significantly higher in males compared to females. Prenatal treatment had no significant effect on social exploration.

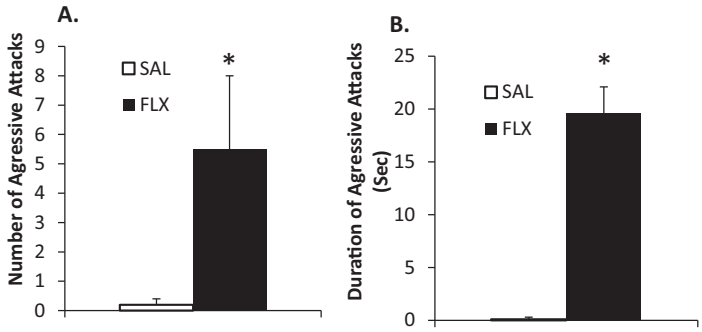

FIGURE 2 Effect of prenatal exposure to saline (SAL) or fluoxetine (FLX) on the number (A) and duration (B) of aggressive attacks in the social exploration test in adult male mice. Prenatal FLX significantly increased the number and duration of aggressive attacks compered to SAL controls. Data are presented as mean ± SD. *Significantly different compared to the corresponding saline group.

(Wimer & Fuller, 1966). Thus, the analysis of aggressive behavior included adult males only. ANOVA with prenatal treatment as a between subjects factor revealed a marginally significant effect for prenatal treatment on the number of aggressive attacks ($F(1,18) = 3.62$, $p = .073$), and the duration of aggressive attacks ($F(1,18) = 4.39$, $p = .051$) exhibited towards the familiar and unfamiliar stimulus partners, indicating that prenatal FLX significantly increased the number and duration of aggressive attacks exhibited by adult male mice in the social memory test (Fig. 3). No preference for attacking an unfamiliar over a familiar target was exhibited. Fisher's exact test was conducted to assess the effect of prenatal treatment on the proportion of male mice that engaged in aggressive behavior. This analysis revealed that there was no significant difference between SAL and FLX males in the proportion of aggressors (5 of 10 SAL males and 6 of 10 FLX males showed aggression, $p > .1$).

Maternal Behavior

Litter Size. Seven of the ten SAL females and eight of the ten FLX females gave birth. Fishers exact test did not show a significant difference in birth incidence between groups ($p > .1$). An independent samples *T*-test between the two treatment groups revealed a marginally significant effect for prenatal treatment on litter size ($t(13) = 2.12$, $p = .054$), suggesting that females prenatally exposed to FLX had a smaller number of pups per litter compared to the control group (SAL(7): $M = 10.14$, SD = 3.38; FLX(8): $M = 6$, SD = 4.07).

Nest Quality. Nest quality scale scores (subjective) and nest size measurements (objective) were analyzed in subjects who gave birth, using an ANOVA with Day of the examination (GD 14, 15, 16) as a within subjects variable, and prenatal treatment (SAL/FLX) as a between subjects variable. A significant interaction was revealed between Day of examination and treatment for nest quality scale ($F(2,22) = 3.97$, $p = .03$) and a marginally significant interaction was found for the nest size measurement ($F(2,22) = 2.98$, $p = .071$). The pattern of behavior was similar in both measurements: on the first Day nest quality was lower in the FLX treatment group compared to the controls, on the second Day quality differences narrowed and on the third Day FLX treatment group exhibited improved

Table 2. Effect of Prenatal Exposure to Saline (SAL) or Fluoxetine (FLX) on the Duration of Social Exploration of a Familiar and Unfamiliar Same-Sex 4-Weeks Old Conspecific in the *Social Memory* Test

		Female		Male	
		Young	Adult	Young	Adult
SAL	Familiar	55.2 (14.8)	62.9 (7.9)	56.7 (8.7)	65.7 (7.5)
	Unfamiliar	56.1 (50.3)	7.4 (7.5)	47.1 (9.0)	70.4 (9.0)
FLX	Familiar	60.3 (6.1)	58.2 (6.3)	54.3 (8.9)	63.1 (4.9)
	Unfamiliar	78.2 (62.3)	11.6 (7.0)	45.7 (6.0)	57.3 (7.0)

Data are presented as mean (SD) in seconds. The total duration of exploration of the stimulus mice was significantly higher in young females compared to young males. Prenatal treatment had no significant effect on the duration of exploration.

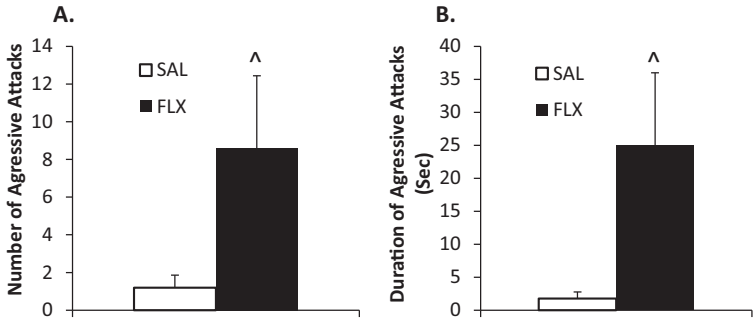

FIGURE 3 Effect of prenatal exposure to saline (SAL) or fluoxetine (FLX) on the number (A) and duration (B) of aggressive attacks in the social memory test in adult male mice. Data depict the total number and duration of aggressive attacks exhibited towards the familiar and unfamiliar stimulus mice. Prenatal FLX increased the number and duration of aggressive attacks compered to SAL controls. Data are presented as mean ± SD. ^Marginally significant difference compared to the corresponding saline group.

nest quality compared to the control group. Further analysis on data gathered on the different days of measurement separately did not reveal a significant difference between treatment groups (Fig. 4A and B).

Pup Retrieval. An analysis of variance with Day of the examination (PND 2, 3, 4) as a within subjects variable, and prenatal treatment (SAL/FLX) as a between subjects variable revealed an interaction between Day of measurement and treatment on retrieval time for the first pup ($F(2,26) = 3.52$, $p = .04$, Fig. 4C), but not for the second pup (Table 3). On PND2, retrieval time for the first pup was longer in the FLX treatment group compared to the control group. On PND3 retrieval time differences narrowed and there was no significant difference between groups, and on PND4 retrieval time was shorter in the FLX treatment compared to the control group. Further analysis on data gathered on the different days of measurement separately did not reveal a significant difference between treatment groups.

DISCUSSION

The present study sought to determine the effects of prenatal FLX exposure on multiple aspects of social interactions in male and female mice. The results demonstrated that prenatal exposure to FLX had no effect on the duration of exploration of a social partner, however, increased aggression in adult male mice. In the presence of a juvenile counterpart, FLX male mice demonstrated significantly higher number and longer duration of aggressive attacks compared to SAL controls. This finding is consistent with several human studies that showed increased aggression following prenatal SSRI exposure (Oberlander et al., 2007, 2010). Animal studies reported variable results. Lisboa et al. (2007) reported that exposure to FLX during pregnancy and lactation had no effect on the number and duration of aggressive attacks in mice. In contrast, Kiryanova and Dyck (2014) reported that exposure of mouse dams to high dose of FLX beginning on gestation Day 15 until postnatal Day 12 increased aggression of male mouse offspring. Along the same lines, it was shown that exposure to fluoxetine on gestation days 13–21 increased foot shock-induced aggression in male offspring (Singh, Jaiswal, Singh, & Bhattacharya, 1998). Studies using other SSRI drugs also showed contradicting results. Exposure of rat dams to citalopram during lactation resulted in reduced aggression in the offspring (Manhaes de Castro et al., 2001), while exposure of mouse dams to paroxetine 4 weeks prior to pregnancy and during pregnancy increased male aggression during cage changing (Coleman, Christensen, Gonzalez, & Rayburn, 1999). Together, these studies show that perinatal exposure to SSRIs is relevant for the development of aggressive behavior; however, the specific outcome may vary depending on differences in drug dosage, timing of administration, procedure of behavioral testing and the species of subjects.

Females prenatally treated with FLX were allowed to breed and litter size and maternal behavior were examined. Prenatal FLX treatment induced a marginal reduction in the number of pups per litter. This finding may suggest that prenatally FLX exposed mothers conceived and gave birth to a smaller number of pups. However, since litter size was recorded on PND1 this reduction may have been due to reduced post-natal survival of pups associated with physiological vulnerability or inadequate maternal care. The latter is

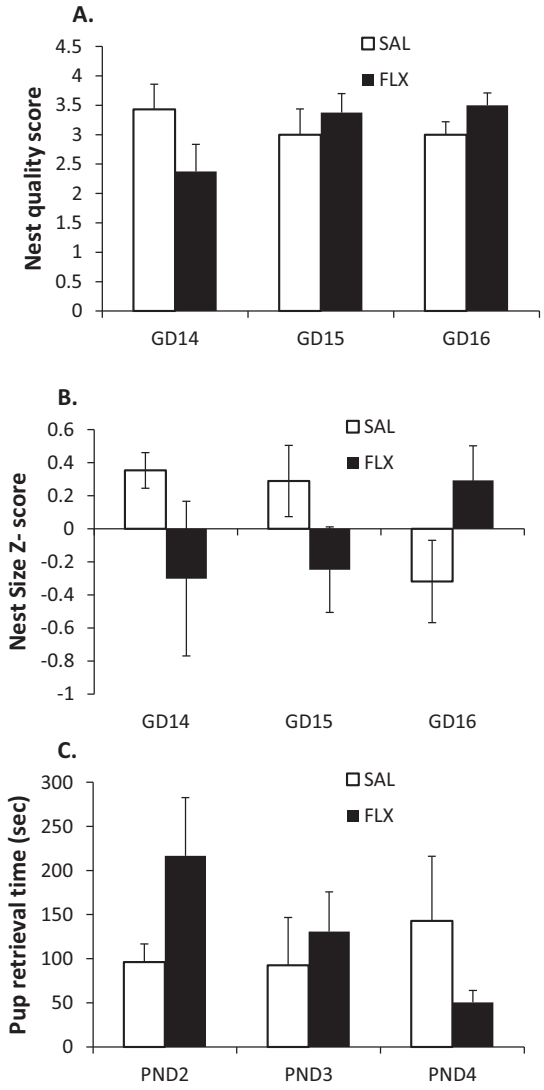

FIGURE 4 Effect of prenatal exposure to saline (SAL) or fluoxetine (FLX) on maternal behavior in adult female mice. Nest quality scale (A) and mean *Z*-score of nest size measurments (B) were assessed on gestations Day (GD) 14–16. Pup retrieval time (C) was assessed on post nataldays (PND) 2–4. Figure depicts the time elaped until retrieval of the first pup into the nest. All maternal behavior pattern show a similar pattern and suggest a delayed emergence of maternal behavior in the FLX-treated group. Data are presented as mean ± SD.

supported by the results of the behavioral tests that demonstrated delayed emergence of maternal behavior in animals prenatally exposed to FLX. Specifically, maternal nest building prior to parturition, and pup retrieval behavior early after birth were altered by prenatal FLX treatment. The two measurements showed a similar response pattern in that during the earlier testing sessions nest quality and pup retrieval were poorer in the FLX group compared to the controls, however, during the later tests the differences narrowed

Table 3. Effect of Prenatal Exposure to Saline (SAL) or Fluoxetine (FLX) on Maternal Behavior in the *Pup Retrieval* Test

	PND2	PND3	PND4
SAL	126.1 (49.2)	114.3 (158.7)	149.3 (201.4)
FLX	236.9 (176.3)	160.5 (138.8)	96.9 (97.8)

Data are presented as mean (SD) in seconds. Pup retrieval time was assessed on post nataldays (PND) 2–4. The table depicts the time elaped until retrieval of the second pup into the nest. Time elapsed until the retrieval of the first pup is presented in Figure 4. Prenatal FLX treatment had no effect on retrieval time of the second pups.

and FLX treatment group exhibited improved maternal care. To the best of our knowledge, this is the first indication of delayed emergence of the instinctive maternal behavior in dams prenatally exposed to SSRIs, and the implications of these findings are yet to be determined. Several brain regions are relevant to maternal behavior in rodents, including the medial preoptic area, ventral tegmental area, hippocampus, and amygdala (Johns et al., 2005). In utero SSRI-related changes in structure or function of these areas may be involved in mediating the observed deficits in maternal behavior (Cabrera-Vera & Battaglia, 1998).

Although the mechanisms of the effects of prenatal SSRI on male aggression and female maternal behavior were not examined in the current study, previous reports suggest a role for oxytocin in these effects. Oxytocin is the most prominent neuro-hormone known to influence and reinforce social and maternal behavior in human and animals (Lieberwirth & Wang, 2014; Rilling & Young, 2014; Walker & McGlone, 2013). In the central nervous system, oxytocin and serotonin neural systems greatly overlap (Dölen, Darvishzadeh, Huang, & Malenka, 2013). Furthermore, evidence suggested a link between serotonin and oxytocin function in the brain. Studies have shown that activation of serotonin receptors promoted the secretion of oxytocin (Van De Kar, Rittenhouse, Li, Levy, & Brownfield, 1995), and SSRIs administration increased levels of oxytocin in adult male rats (Uvnäs-Moberg, Björkstrand, Hillegaart, & Ahlenius, 1999). A recent study further reported that blocking serotonin neurotransmission impaired social reinforcement signal received throughout the oxytocin system (Dölen et al., 2013). Prenatal SSRI exposure was reported to affect the development of serotonergic pathways (Hanley & Oberlander, 2012). The subsequent changes in serotonergic function may cause further changes in the function of oxytocin pathways, resulting in the observed changes in behavior.

In the present study, most of the effects of prenatal FLX exposure appeared in adult animals and young

mice were hardly affected. Previous studies indicated that the effects of perinatal FLX exposure often vary with age. Perinatal FLX induced anxiety- and depressive like behavior in young animals only (Francis-Oliveira et al., 2013). Furthermore, we have recently reported age-dependent changes in immune function following prenatal FLX exposure (Avitsur et al., 2015a). Age-related changes in the response to perinatal FLX may be associated with changes in serotonin neurotransmission and function throughout life. An earlier report showed that perinatal exposure to SSRIs decreased serotonin content in the frontal cortex in prepubescent rats, while reducing serotonin content in the midbrain of adults (Cabrera-Vera, Garcia, Pinto, & Battaglia, 1997). Prenatal FLX exposure also altered the density of serotonin transporters in sub-regions of the hypothalamus, hippocampus, and amygdala in prepubescent offspring rats, while serotonin transporter density in these brain regions was unaltered in adults (Cabrera-Vera & Battaglia, 1998). The present findings support the notion that the effects of prenatal SSRI vary according to the developmental stage of the brain and add to the understanding of the functional consequences and implications of prenatal alterations in brain serotonergic systems.

In the current study, two recognition memory tests were conducted (Bevins & Besheer, 2006). In the object memory test, mice showed a significant preference for an unfamiliar over a familiar object, demonstrating that the memory of the familiar object was intact. In the social memory test, mice did not show preference for the unfamiliar over the familiar juvenile conspecific; however, these findings may have been influenced by the high levels of aggression observed and may therefore not accurately represent social memory. In both tests, in utero FLX exposure had no effect on the preference for the unfamiliar object or social counterpart, indicating that these aspects of memory function were not affected by prenatal FLX. Reports regarding the long-term outcomes of prenatal SSRI exposure on learning and memory are not abundant. Preschool children prenatally exposed to SSRIs displayed normal intelligence and language skills (Nulman et al., 1997). In rats, prenatal FLX was associated with improved learning and memory in the Morris water maze (Bairy et al., 2007), and with sensorimotor learning deficits (Lee, 2009). Thus, additional studies are necessary to delineate the possible effects of prenatal SSRIs on cognitive function, learning, and memory.

The current study adds to an increasing number of reports indicating that prenatal exposure to SSRIs is associated with lasting developmental changes. One limitation of this study is that cross-fostering procedure was not conducted. Although many studies utilized procedures similar to the current study (e.g., Avitsur et al., 2015b; Bairy et al., 2007; Lisboa et al., 2007; Pawluski et al., 2012), our procedure does not allow separating gestational effects from possible postpartum consequences of exposure to fluoxetine associated with changes in maternal care. Another limitation of this study is that in the clinic SSRIs are normally used in the context of maternal depression, however, in the present study FLX was given to healthy pregnant dams. Maternal depression, by itself, has a large negative impact on offspring development and well-being (Reddy, 2010; Yonkers et al., 2009). Maternal mental illness during pregnancy were associated with changes in the development of essential body systems, slower fetal growth, increased risk for premature delivery and greater incidence of low birth weight (Field, Diego, & Hernandez-Reif, 2006, Field, 2011). Thus, in order to fully determine the advantages of antidepressant medication over the untreated psychiatric disorder it is important to distinguish between the effects of maternal illness from those of the SSRIs medication. It is our hope that additional studies will contribute to the understanding of the effects of prenatal environment on the responses to life challenges, and assist in providing better care for women suffering from mood disorders and their offspring.

NOTES

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