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Research report

Perinatal fluoxetine treatment and dams' early life stress history alter affective behavior in rat offspring depending on serotonin transporter genotype and sex

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ABSTRACT

Many women diagnosed with a major depression continue or initiate antidepressant treatment during pregnancy. Both maternal stress and selective serotonin inhibitor (SSRI) antidepressant treatment during pregnancy have been associated with changes in offspring behavior, including increased anxiety and depressive-like behavior. Our aim was to investigate the effects of the SSRI fluoxetine (FLX), with and without the presence of a maternal depression, on affective behavior in male and female rat offspring. As reduced serotonin transporter (SERT) availability has been associated with altered behavioral outcome, both offspring with normal ($SERT^{+/+}$) and reduced ($SERT^{+/-}$) SERT expression were included. For our animal model of maternal depression, $SERT^{+/-}$ dams exposed to early life stress were used. Perinatal FLX treatment and early life stress in dams (ELSD) had sex- and genotype-specific effects on affective behavior in the offspring. In female offspring, perinatal FLX exposure interacted with SERT genotype to increase anxiety and depressive-like behavior in $SERT^{+/+}$, but not $SERT^{+/-}$, females. In male offspring, ELSD reduced anxiety and interacted with SERT genotype to decrease depressive-like behavior in $SERT^{+/-}$, but not $SERT^{+/+}$, males. Altogether, $SERT^{+/+}$ female offspring appear to be more sensitive than $SERT^{+/-}$ females to the effects of perinatal FLX exposure, while $SERT^{+/-}$ male offspring appear more sensitive than $SERT^{+/+}$ males to the effects of ELSD on affective behavior. Our data suggest a role for offspring SERT genotype and sex in FLX and ELSD-induced effects on affective behavior, thereby contributing to our understanding of the effects of perinatal SSRI treatment on offspring behavior later in life.

1. Introduction

Being the leading cause of disability worldwide, major depressive disorder (MDD) is affecting approximately 4.4 % of the global population [1], with a higher prevalence in women. During pregnancy, hormonal changes can increase vulnerability for the onset or return of depression, especially during the 2nd and 3rd trimester [2]. Consequently, one in five pregnant women experiences symptoms of a depression [3], while approximately 4–7.5 % of women actually suffer from MDD during pregnancy [3–5]. Being depressed during pregnancy has been associated with several adverse pregnancy outcomes, including poor neonatal adaptation, premature delivery and reduced fetal growth (reviewed in [6]). On the long term, a maternal depression has the potential to alter neurobehavioral development of the offspring. For instance, being depressed during pregnancy has been associated with attention and emotional problems in 4-year olds [7,8], increased anxiety in 6-year olds [9], increased internalizing in 12-year olds [10], and an increased risk to develop a depression during adolescence or adulthood [11,12]. When pregnant women are suffering from a moderate to severe depression, antidepressant treatment is often recommended [13]. Selective serotonin reuptake inhibitors (SSRIs) are the most frequently prescribed class of antidepressants during pregnancy, with prescription up to 3 % in Europe and even up to 13 % in the United States [14–16]. By blocking the serotonin transporter (SERT), SSRIs prevent reuptake of synaptic serotonin back into the presynaptic cell, resulting in increased extracellular serotonin levels and prolonged serotonergic signaling. SSRIs cross the placenta and have been found in human breast milk, exposing not only the mother but also the developing child to the SSRIs [17,18]. Whereas serotonin is an important regulator of mood during adulthood, serotonin acts as a neurotrophic

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factor modulating important neurodevelopmental processes during early brain development [19–21]. Consequently, changes in serotonin levels during neurodevelopment are not without risks for the developing child. For instance, SSRI treatment during pregnancy has been associated with increased internalizing and anxious behaviors in 3 and 6 year olds [22,23], and an increased risk for attention-deficit hyperactivity disorder [24]. Furthermore, SSRI use during pregnancy has repeatedly been linked to an increased risk for autism spectrum disorder [25–28], whereas others did not find this link or suggest that this increased risk is caused by the depression itself rather than the SSRIs [29,30]. Also, maternal child abuse was shown to elevate the risk for autism in the offspring [31]. Together this indicates that in clinical studies it is hard to directly dissociate the effects of the maternal depression from the SSRI treatment and when controlled for the severity of the maternal depression, associations of prenatal SSRI treatment with behavioral problems in the child do not always persist. Fortunately, preclinical studies can provide a distinct indication as to whether SSRI exposure during development, both separately as well as combined with maternal stress, alters behavior of the offspring later in life. However, most preclinical studies have investigated the effects of SSRI treatment using only healthy rodents, while studying the effects of perinatal SSRI treatment in the presence of a stressed mother is more clinically relevant. In rodents, it has been shown repeatedly that both perinatal SSRI exposure and prenatal stress can result in increased anxiety and depressive-like behavior in the offspring, here referred to as affective behavior (reviewed in [32,33]. Studies investigating the offspring on the neurobehavioral effects of SSRI exposure during the perinatal period in combination with an animal model of depression have been increasing in number (e.g. [34–37]. However, only few of those studies focused on the effects of SSRI treatment and maternal stress on affective behavior in the offspring [34,36]. In the present study, we investigated the effects of perinatal SSRI exposure with and without the presence of maternal stress, on offspring affective behavior, more specifically anxiety and depressive-like behavior. We used an animal model of depression, which utilizes heterozygous serotonin transporter knockout ($SERT^{+/-}$) dams exposed to early life stress (ELSD). We have previously shown that this procedure induces anhedonia, reflecting depressive-like behavior and decreased expression of neuronal growth factor levels in brain areas relevant to depression. [38]. These characteristics display the face validity of the model with symptoms already present prior to conception. Throughout pregnancy and lactation, dams exposed to early life stress were treated with the SSRI fluoxetine (FLX) or vehicle to investigate potential long-term alterations in affective behavior in both male and female offspring. Since both pharmacological blockade of the SERT with SSRIs and lifelong reduced SERT expression alter behavioral development in rodents [39–41], both $SERT^{+/+}$ and $SERT^{+/-}$ offspring were investigated. In humans, a reduction in SERT expression has been associated with increased affective behavior after stressful life events [42], but also with poorer treatment response [43]. Therefore, we expected offspring SERT genotype to interact with FLX exposure and ELSD. Furthermore, since neonatal FLX exposure increases affective behavior such as anxiety and depressive-like behavior in rodents (e.g. [44,45], we expected FLX exposure to increase affective behavior in the present study as well. Similar to effects of prenatal stress [46], we also expected ELSD to increase anxiety and depressive-like behavior in the offspring.

2. Methods

2.1. Animals

Wistar rats were housed in Makrolon type 3 cages (38.2 × 22.0 × 15.0 cm) during individual housing or Makrolon type 4 cages (55.6 × 33.4 × 19.5 cm) during social housing. Animals were given ad libitum access to food RMH-B, AB Diets; Woerden, the Netherlands) and tap water and were maintained on a reversed 12 h light/dark cycle (lights off at 11:00 a.m.). Environmental enrichment was provided as a wooden gnawing stick (10 × 2 × 2 cm) and nesting material (Envirodri®). All breeding occurred in our own animal facility and experimental procedures were approved by the Groningen University Committee of Animal experiments (DEC6936A).

2.2. Early life stress in dams

Serotonin transporter knockout rats (Slc6a41Hubr) were bred crossing $SERT^{+/-}$ females with $SERT^{+/-}$ male rats, resulting in offspring of three genotypes ($SERT^{+/+}$, $SERT^{+/-}$ and $SERT^{-/-}$). These pups (future dams) were randomly assigned to either the control group (CTR) or the early life stress group (ELS). ELS consisted of maternally separating both male and female pups of all SERT genotypes as a whole litter for 6 h a day from postnatal day (PND)2 − 15. As a control (CTR), pups were taken away during this period for 15 min and handled briefly. Pups were weaned at PND21 and socially housed with same treated, same sex pups from different litters. As we have previously shown that adult $SERT^{+/-}$ females exposed to ELS show depressive-like behavior [38] they were used as an animal model for maternal depression.

2.3. Perinatal fluoxetine treatment

In total, 134 adult female $SERT^{+/-}$ (65 CTR and 69 ELS) and 47 male $SERT^{+/+}$ Wistar rats were used for breeding and fluoxetine treatment. When in estrus, determined by measuring vaginal wall impedance (model MK-11, Muromachi, Tokyo, Japan), a female was placed with a male for a 24 -h period (Gestational day 0: G0) and housed singly afterwards. Females from both CTR and ELS groups were randomly assigned to the fluoxetine or vehicle group. From G0 until weaning of the pups at PND21 (a total of 6 weeks), dams were weighed and treated daily at 11:00 a.m. with either 10 mg/kg fluoxetine (FLX, Fluoxetine 20 PCH, Pharmachemie BV, the Netherlands) or a vehicle (VEH, Methylcellulose 1 %, Sigma Aldrich Chemie BV, Zwijndrecht, the Netherlands) using flexible PVC feeding tubes (40 cm length, Vygon, Valkenswaard, the Netherlands) for oral gavage. With these feeding tubes, animals can be orally treated by gently picking up the animal without restraining them and minimizing stress. The dose of fluoxetine was based on comparison to human dosing studies [47,48] and previous studies performed in our laboratory [49,50]. Four groups of dams were used: (1) Control dams + vehicle treatment (CTR-VEH) (n = 24), (2) Control dams + fluoxetine treatment (CTR-FLX) (n = 41), (3) ELS in dams + vehicle treatment (ELSD-VEH) (n = 21) and (4) ELS in dams + fluoxetine treatment (ELSD-FLX) (n = 48). Near the end of pregnancy, dams were checked twice a day (9:00 a.m. and 5:00 p.m.) for pup delivery (PND 0). At PND21, offspring were weaned and ears punched for individual recognition and genotyping as described previously [51]. Both wildtype ($SERT^{+/+}$) and heterozygous ($SERT^{+/-}$), male and female offspring were used in this study, coming to a total of 16 offspring groups (Fig. 1). Pups were housed in groups of 3–5 with same-sex and same-treated animals in Makrolon type 4 cages under the same conditions as the dams. Due to unexpected high mortality rates in dams and offspring (about 25 %) from FLX groups, we ended up with less dams and litters for offspring groups than before start of treatment (see [49] for more details on dam and pup mortality). In total, 4 batches were used in this study. Per litter, part of the offspring was used for the current study, while other pups in litters from the first 3 batches were used for parallel experiments including social play, social interaction [49], aggressive and sexual behavior (Houwing et al., submitted). In the end, offspring for the current study came from 60 litters (CTR-VEH: 18, CTR-FLX: 17, ELSD-VEH: 11, ELSD-FLX: 14).

2.4. Behavioral testing

Male and female wildtype ($SERT^{+/+}$) and heterozygous ($SERT^{+/-}$)

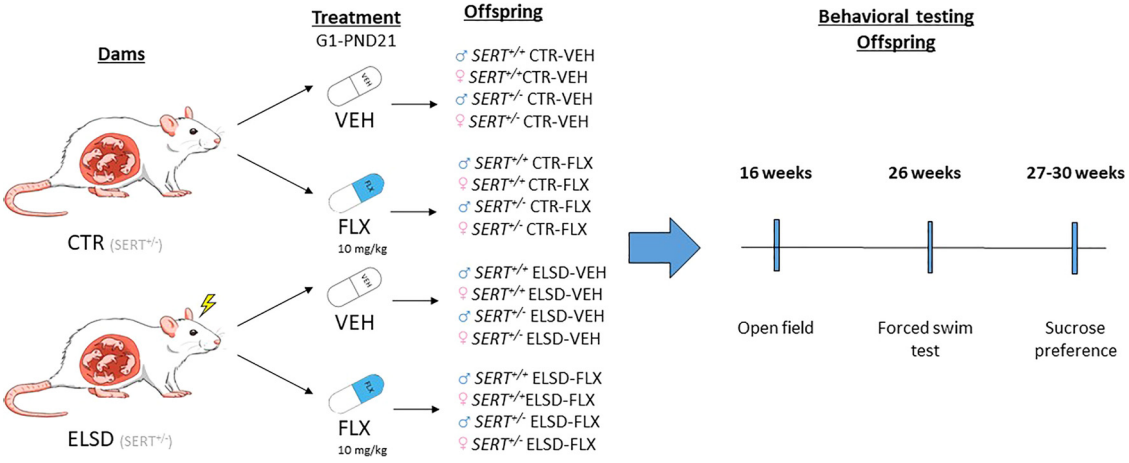

Fig. 1. Schematic timeline of FLX treatment and offspring behavioral testing.

offspring were tested for changes in affective behavior during adulthood, including anxiety in the open field, stress coping in the forced swim test and depressive-like behavior in the sucrose preference test. Testing occurred during the active dark phase of the animals, between 12:00 and 5:00 p.m.

2.4.1. Open Field (OF)

At 16 weeks of age, animals were tested for anxiety-like behavior in the OF. The OF consisted of an open square arena (100 × 100 × 40 cm), with a black floor and walls made of wood. The arena was subdivided into a center (square area of 60 × 60 cm) and outer area near the walls (20 cm wide). Animals were tested under dim light conditions (2.5 lx). At the beginning of each trial, an animal was placed in a corner and exploration was recorded on video for 10 min. Using automated animal tracking software (EthoVision XT, Noldus, The Netherlands), the total distance moved (m) and the time the animal spent in the center of the OF were calculated. Between trials, the OF was cleaned using a 70 % ethanol solution to remove olfactory cues.

2.4.2. Forced swim test (FST)

At 26 weeks of age animals were tested for stress coping in the FST. Animals were placed in cylindrical Plexiglas tanks (50cm × 18cm diameter), filled with 30 cm of water (22 ± 1 °C). On the first day, animals were placed in individual tanks for 15 min. Upon removal, animals were dried with a towel and placed in a cage on a heating mat to recover. On the second day, exactly 24 h later, animals were re-tested for 5 min. Animals were tested under dim light conditions (2.5 lx). Animals that were simultaneously tested were visually separated from animals in the other tanks. Females were checked for estrous cycle phase (model MK-11, Muromachi, Tokyo, Japan) on the first day of the FST and were not tested if they were in estrus. The FST was recorded on video and percentage of time spent in mobility and immobility was scored by one observer blind for treatment (The Observer XT 11.0, Noldus, The Netherlands). Active climbing, swimming and diving were scored as mobility, while immobility was defined as making no movements for at least 2 s or making only those movements that were necessary to keep the nose above water. Slightly moving of the paws or support by pressing paws against the wall of the tank was still considered immobility. Tanks were cleaned and refilled between trials.

2.4.3. Sucrose preference test (SPT)

At 27–30 weeks of age, animals were tested for their preference for a sucrose solution over water. First, animals were individually housed and habituated with two water bottles, one on each side of the cage, for 3 consecutive days. Following habituation, animals were presented with one water bottle and one bottle containing a sucrose solution for 24 h on alternating days. On the other days two bottles of water were presented. Starting with a 0.1 % sucrose solution, the sucrose concentration gradually increased with 0.1 % each sucrose day (0.1 %–1 %). After that, sucrose concentrations increased with 1 % (1 %–4 %). In total, 13 different sucrose concentrations were given over a period of 4 weeks. Sucrose bottle locations on the cage were alternated on sucrose days to prevent spatial bias. Fluid consumption (grams) was determined daily and animals were weighed on sucrose days. The preference for sucrose above water was calculated ((sucrose solution intake (g)/total fluid intake (g)) x 100 %). In addition, the actual sucrose intake in mg per gram rat was calculated and corrected for body weight ((((sucrose solution intake (g)/100)*sucrose concentration (%))/body weight (g))*1000).

2.5. Statistical analysis

The Statistical Package for the Social Sciences software version 22 (SPPS Inc., IBM SPSS Statistics, Chicago) was used to perform statistics. We previously found sex specific effects of perinatal FLX exposure in the offspring [49], and again in the present study. A four-way (FLX x ELSD x genotype x sex) ANOVA showed sex differences in the FST, with males spending a higher percentage of time being immobile than females ($F(1,151) = 4.953$, $p < .05$). Also, sex differences in the SPT were found, with females showing on average a higher sucrose preference ($F(1,151) = 8.517$, $p < .01$) and a higher sucrose intake ($F(1,151) = 15.591$, $p < .001$) than males. Therefore, data was split and ANOVAs were performed per sex. For the OF and FST, a three-way ANOVA was performed per sex to determine main and/or interaction effects of FLX, ELSD and genotype. For the SPT, a repeated measures ANOVA with FLX, ELSD and genotype as between subjects factor and sucrose concentration as within subjects factor was performed per sex. When appropriate, Fisher LSD was used *post hoc* to correct for multiple comparisons. All statistics were two-tailed with values of $p \leq .05$ being considered significant. All data and all figures are presented as means ± standard error of the mean (SEM).

3. Results

3.1. Open Field (OF)

Effects of perinatal FLX exposure and ELSD on anxiety-like behavior in $SERT^{+/+}$ and $SERT^{+/-}$ offspring were assessed using the OF. Regarding the total distance moved in the OF, perinatal FLX exposure and genotype interacted in both male and female offspring, regardless of ELSD (males: $F(1,75) = 4.856$, $p < .05$, Fig. 2A; females: $F(1,76) = 14.420$, $p < .001$, Fig. 2B). For males, *post hoc* analysis showed that

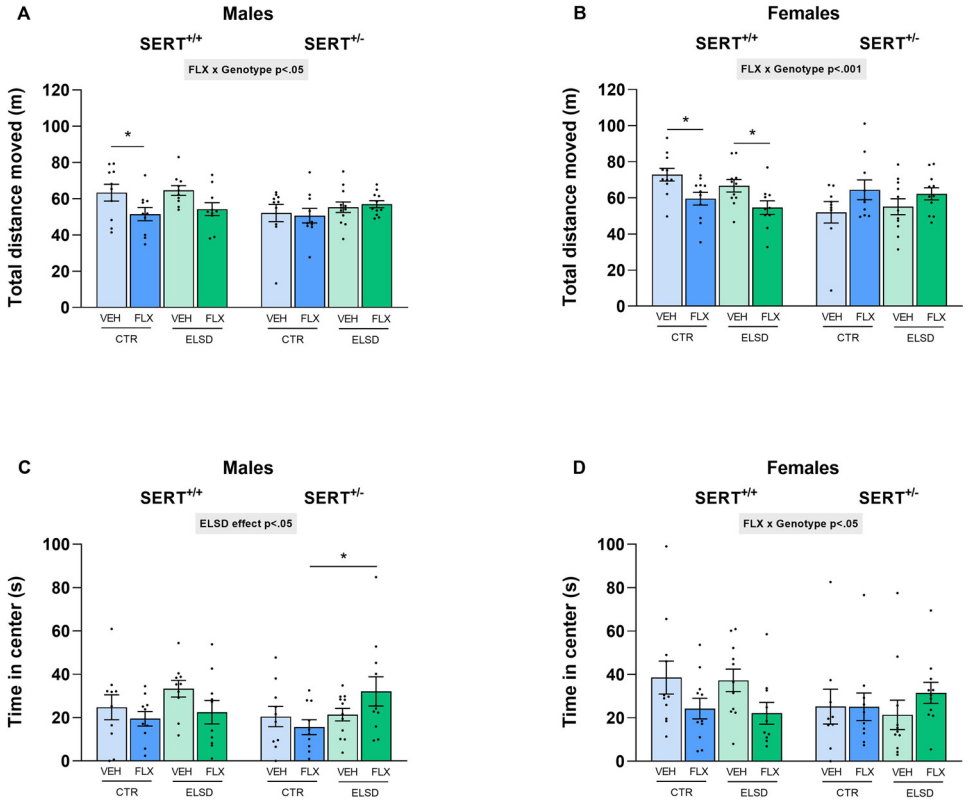

Fig. 2. Effects of perinatal FLX exposure and ELSD on male (A) and female (B) total distance moved and male (C) and female (D) time spent in the center of the open field. Figures show mean ± SEM. *p ≤ .05, n = 9-12 per group.

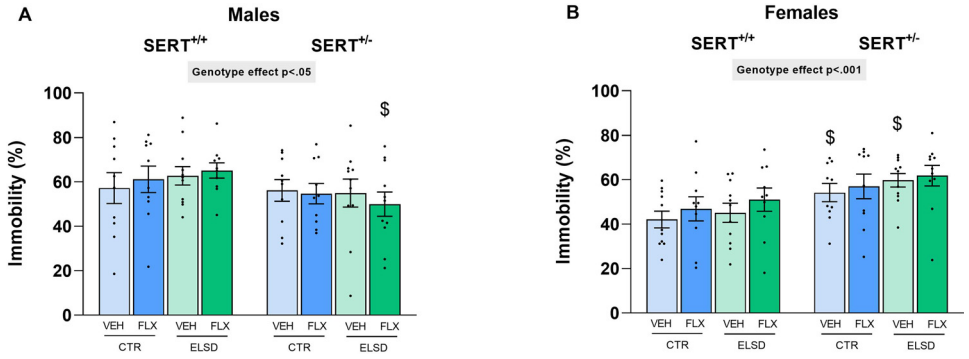

Fig. 3. Effects of perinatal FLX exposure and ELSD on percentage of time spent immobile in the FST for males (A) and females (B). Figures show mean ± SEM, $ p < .05 (vs $SERT^{+/+}$), n = 9-12 per group.

$SERT^{+/+}$ CTR-FLX offspring had a lower total distance moved in the OF compared to $SERT^{+/+}$ CTR-VEH offspring ($p < .05$, Fig. 2A). In addition, $SERT^{+/+}$ ELSD-FLX male offspring tended to have a lower total distance moved compared to $SERT^{+/+}$ ELSD-VEH offspring ($p = .06$, Fig. 2A). For females, $SERT^{+/+}$ CTR-FLX offspring had a lower total distance moved compared to $SERT^{+/+}$ CTR-VEH offspring ($p < .05$, Fig. 2B). Furthermore, $SERT^{+/+}$ ELSD-FLX female offspring had a lower total distance moved compared to $SERT^{+/+}$ ELSD-VEH offspring ($p < .05$, Fig. 2B). On the contrary, a tendency towards a higher total distance moved was found for $SERT^{+/-}$ CTR-FLX female offspring compared to $SERT^{+/-}$ CTR-VEH offspring ($p = .08$, Fig. 2B). For the time the animals spent in the center of the OF, a main effect of ELSD was found in male offspring, with ELSD offspring significantly spending more time in the center, regardless of FLX exposure and genotype ($F(1,75) = 4.764$, $p < .05$, Fig. 2C). *Post hoc* analysis showed that $SERT^{+/-}$ ELSD-FLX male offspring significantly spent more time in the center of the OF, compared to $SERT^{+/-}$ CTR-FLX male offspring ($p < .05$, Fig. 2C). For female offspring, an interaction between FLX exposure and genotype was found for the time spent in the center ($F(1,76) = 5.175$, $p < .05$, Fig. 2D), with effects found only in $SERT^{+/+}$ female offspring. *Post hoc* analysis showed that $SERT^{+/+}$ CTR-FLX and $SERT^{+/+}$ ELSD-FLX female offspring tended to spent less time in the center of the OF, compared to $SERT^{+/+}$ CTR-VEH offspring (vs. CTR-FLX: $p = .09$, vs. ELSD-FLX: $p = .08$, Fig. 2D). Likewise, $SERT^{+/+}$ ELSD-FLX female offspring tended to spent less time in the center of the OF, compared to $SERT^{+/+}$ ELSD-VEH offspring ($p = .06$, Fig. 2D).

3.2. Forced swim test (FST)

To assess effects of FLX exposure and ELSD on stress coping, $SERT^{+/+}$ and $SERT^{+/-}$ offspring were assessed with the FST. For male offspring, a significant main effect of genotype was found, with $SERT^{+/-}$ offspring spending a lower percentage of time being immobile in the FST compared to $SERT^{+/+}$ offspring, regardless of FLX exposure or

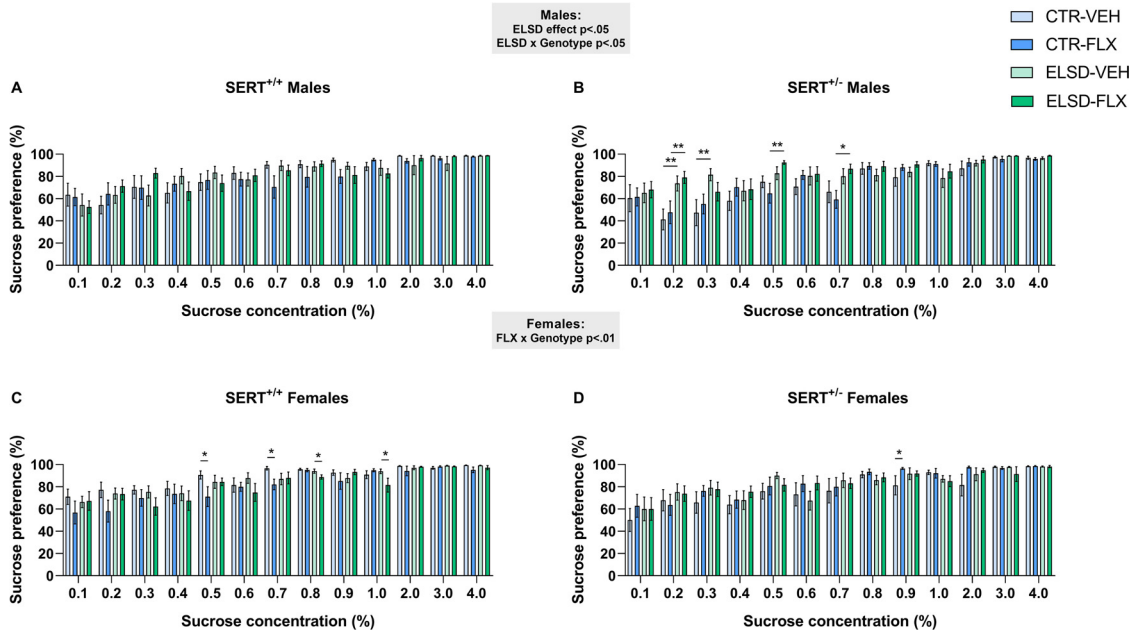

Fig. 4. Effects of perinatal FLX exposure and ELSD on sucrose preference in SERT$^{+/+}$ (A) and SERT$^{+/-}$ (B) males, and SERT$^{+/+}$ (C) and SERT$^{+/-}$ (D) females. Figures show mean ± SEM. *p ≤ .05, **p < .01, n = 9-12 per group.

ELSD ($F(1,75) = 4.062$, $p < .05$, Fig. 3A). *Post hoc* analysis revealed that SERT$^{+/-}$ ELSD-FLX offspring spent a significantly lower percentage of time being immobile in the FST compared to SERT$^{+/+}$ ELSD-FLX offspring ($p < .05$, Fig. 3A). For female offspring a significant main effect of genotype was found as well, with SERT$^{+/-}$ offspring spending a higher percentage of time being immobile in the FST compared to SERT$^{+/+}$ offspring, regardless of FLX exposure or ELSD ($F(1,76) = 13.870$, $p < .001$, Fig. 3B). More specific, *post hoc* analysis showed that SERT$^{+/-}$ CTR-VEH offspring spent a higher percentage of time being immobile in the FST compared to SERT$^{+/+}$ CTR-VEH offspring ($p < .05$, Fig. 3B). Also, SERT$^{+/-}$ ELSD-VEH treated female offspring spent a higher percentage of time being immobile in the FST compared to SERT$^{+/+}$ ELSD-VEH female offspring ($p < .05$).

3.3. Sucrose preference test

To assess effects of FLX exposure and ELSD on depressive-like behavior, SERT$^{+/+}$ and SERT$^{+/-}$ offspring were tested using the sucrose preference test. Both male and female offspring showed an increasing preference for a sucrose solution with an increasing sucrose concentration (males: $F(6.006, 450.482) = 18.400$, $p < .001$, Fig. 4A-B; females: $F(5.830, 443.100) = 19.840$, $p < .001$, Fig. 4C-D). For male offspring an interaction between ELSD and genotype on sucrose preference was found, regardless of FLX exposure ($F(1,75) = 4.327$, $p < .05$, Fig. 4A-B). In addition, a main effect of ELSD was found, with ELSD increasing sucrose preference in male offspring ($F(1,75) = 6.732$, $P < .05$, Fig. 4A-B). *Post hoc* analysis showed increasing effects of ELSD on sucrose preference in SERT$^{+/-}$ male offspring at 0.2, 0.3, 0.5 and 0.7 % sucrose solution compared to CTR SERT$^{+/-}$ male offspring (Fig. 4B), while no effects were observed in SERT$^{+/+}$ male offspring (Fig. 4A). For female offspring, an interaction between FLX exposure and genotype on sucrose preference was found ($F(1,76) = 10.002$, $P < .01$, Fig. 4C-D). *Post hoc* analysis revealed that FLX exposure significantly lowered the sucrose preference at 0.5, 0.7, 0.8 and 1.0 % sucrose solution in SERT$^{+/+}$ offspring (Fig. 4C), while FLX exposure increased sucrose preference at 0.9 % sucrose solution in SERT$^{+/-}$ offspring compared to CTR SERT$^{+/-}$ offspring (Fig. 4D). Regarding sucrose intake, both male and female offspring increased their sucrose intake with increasing sucrose concentrations (males: $F(4.254, 319.082) = 272.212$, $p < .001$; females: $F(3.573, 271.535) = 279.817$, $p < .001$, data not shown). Furthermore, a main effect of genotype was found in female offspring, with SERT$^{+/-}$ offspring having a lower sucrose intake compared to SERT$^{+/+}$ female offspring ($F(1,76) = 5.799$, $p < .05$, data not shown). No other main and/or interaction effects of perinatal FLX exposure, ELSD and genotype were found in males or females.

4. Discussion

In the present study, we investigated the long-term effects of perinatal FLX treatment and ELSD, both separately and combined, on affective behavior in adult male and female offspring with normal and diminished SERT expression. Our main findings demonstrate that perinatal FLX exposure and ELSD alter affective behavior in offspring, but effects are genotype and sex dependent. FLX exposure increased anxiety-like behavior in female offspring, but only in SERT$^{+/+}$ female offspring and due to a shorter distance covered in the OF in the SERT$^{+/+}$ CTR-FLX offspring compared to CTR-VEH offspring, these effects might be activity dependent. Furthermore, FLX exposure interacted with genotype to increase depressive-like behavior in SERT$^{+/+}$ female offspring. Opposite to the effects of FLX exposure, ELSD had reducing effects on anxiety- and depressive-like behavior. ELSD decreased anxiety-like behavior in SERT$^{+/-}$ male offspring exposed to FLX. Also, ELSD interacted with offspring genotype to decrease depressive-like behavior in SERT$^{+/-}$ male offspring, but not in SERT$^{+/+}$ male offspring. All take together, our findings show that SERT$^{+/+}$ female offspring are more sensitive than SERT$^{+/-}$ female offspring to the effects of perinatal FLX exposure, while SERT$^{+/-}$ male offspring are more sensitive than SERT$^{+/+}$ male offspring to the effects of ELSD on affective behavior.

4.1. Effects of perinatal FLX exposure on anxiety- and depressive-like behavior

In line with our expectations, perinatal FLX treatment altered affective behavior by increasing anxiety- and depressive-like behavior in the offspring. Both male and female SERT$^{+/+}$ CTR-FLX offspring covered a shorter distance in the OF compared to CTR-VEH offspring,

indicative of reduced motor activity. Similarly, female SERT$^{+/+}$ ELSD-FLX offspring were less active in the OF than ELSD-VEH offspring. The finding that perinatal FLX exposure reduces activity in the OF has been found repeatedly when FLX was administered directly to the offspring [39,45,52–55]. Surprisingly, in previous studies that administered FLX directly to the dam, offspring did not show altered activity levels [34,37,44,48,56–59]. These differences might appear due to the timing of treatment. For example, an earlier study performed by our lab [48] showed no effect of FLX treatment during development on OF activity in rat offspring. This study differed from the current study as treatment occurred only during gestation, while the dams in the present study were treated during both gestation and lactation. The studies that found reduced OF field activity after FLX was administered directly to offspring also cover the postnatal period. This is similar to our study, indicating that the period of treatment is of importance. We also found that SERT$^{+/+}$ female offspring tended to reduce the time they spent in the center of the OF. However, as we simultaneously found reduced activity in the OF, we cannot claim this reflects increased anxiety-like behavior. Mixed results are found on anxiety levels in the OF after developmental FLX exposure. Some studies show increased anxiety-like behavior, as seen by less visits to the center of the OF, in male rats [45,56] and mice [52]. Other studies find no effect of developmental FLX exposure on anxiety in the OF at all in male [34,53,55,60] or female [44,57,59] rodents. Interestingly, in the elevated plus maze - another test frequently used to assess anxiety levels in rodents - anxiety levels in offspring from FLX treated dams were not altered [39,40,45,48,52,58,61] or anxiety-like behavior was even decreased [34,59,60]. Discrepancies between studies could be the result of many factors, including differences in species, dosage, administration route and treatment period (pregnancy versus lactation). Rebello and colleagues showed that the early postnatal period (P2−11) is a critical time period in mice during which FLX exposure results in persistent changes in anxiety levels [62]. If any, our results only show a small non-significant increase in anxiety, which might be due to the P2−11 treatment period.

To assess the effects of perinatal FLX exposure on stress coping and depressive-like behavior, we subjected offspring to the FST and a sucrose preference test. In both males and female offspring, we found no effects of perinatal FLX exposure on immobility in the FST. In line with our results, other studies also found no effect of perinatal FLX exposure on immobility time in the FST in neither male [37,44,48,52,58] nor female [52] rodents. In contrast, increased immobility time [44,45,58,62], as well as decreased immobility time [53] after early life FLX exposure has been found as well, indicating that FLX may alter stress coping responses under some conditions. Again, studies varied greatly in their experimental design, possibly contributing to the inconsistencies found between studies. Whether altered stress-coping is indicative of depressive-like behavior remains to be investigated. To measure depressive-like behavior in the offspring we used a sucrose preference test, which assesses anhedonia, a well-known symptom of depression. By doing so, we found an effect of perinatal FLX exposure on sucrose preference in female offspring. In fact, FLX exposure interacted with SERT offspring genotype, with SERT$^{+/+}$ female offspring exposed to FLX showing a lower preference for sucrose compared to VEH exposed female offspring, while SERT$^{+/-}$ female offspring were barely affected. Even so, post hoc testing showed that this effect of FLX is not consistent over the different sucrose concentrations. Furthermore, male offspring were not sensitive to perinatal FLX exposure regarding sucrose preference and intake, as no differences were found between CTR and FLX exposed rats. This is not surprising as others have shown similar results in rodents [37,48,63]. Anhedonic behavior as a result of perinatal FLX exposure is something that has scarcely been observed in adult males [62] and has not been found before in adult female rodents [63]. However, studies using the sucrose preference test to assess anhedonia after developmental FLX exposure are limited to begin with and therefore findings need to be replicated. Even so, it appears that rat offspring are a bit more anxious and depressive-like, which agrees with clinical studies, where SSRI treatment during pregnancy has been associated with increased internalizing and anxious behaviors in 3 and 6 year olds [22,23], and an increased risk for attention-deficit hyperactivity disorder in the offspring [24].

All in all, a main effect of perinatal FLX exposure resulted in anhedonia in SERT$^{+/+}$ females, independent of ELSD, reflecting a small increase in depressive-like behavior when compared to CTR SERT$^{+/+}$ females. Together, our data suggest that FLX exposure during the perinatal period has long-term effects on affective behavior, particularly in SERT$^{+/+}$ female offspring.

4.2. Effects of early life stress in dams on offspring anxiety- and depressive-like behavior

In the present study we used SERT$^{+/-}$ dams exposed to ELS (ELSD) as an animal model for maternal depression. Against our expectations, ELSD resulted in more time spent in the center of the OF and an increased preference for a sucrose solution in SERT$^{+/-}$ male offspring, suggesting reduced anxiety- and depressive-like behavior. No differences in stress coping in the FST were found. At the same time, no effects of ELSD were found in any female offspring, or in SERT$^{+/+}$ male offspring for any of the behavioral tests. While rat offspring from depressive-like dams appear to be *less* anxious and depressive-like, a maternal depression in humans has been associated with increased anxiety in 6-year olds [9], increased internalizing behavior in 12-year olds [10] and an increased risk to develop a depression during adolescence or adulthood [11,12]).

Our findings oppose many studies where pre-gestational and prenatal stress mainly result in increased anxiety- and depressive-like behavior in rodents [46]. The observation that our dams show increased depressive-like behavior, while the offspring of these dams show a decrease in depressive-like behavior, might be due to the stress protocol we applied to our dams early in life. Unlike previous studies, we used an animal model for maternal depression consisting of exposing SERT$^{+/-}$ females to maternal separation from postnatal day 2–15. Even though these females show depressive-like behavior (anhedonia) during adulthood [38], we did not investigate possible effects of early life stress on maternal behavior and maternal care in our dams during the postnatal period. The idea that offspring exposed to adverse rearing environments (maternal stress and fluoxetine treatment) are more vulnerable for subsequent behavioral problem builds on the Belsky theory [64,65]. This evolutionary theory is built on the hypothesis that some individuals are more sensitive to environmental influences (both positive and negative), especially rearing effects. Maternal attachment might play an important factor here. Since depressive-like behavior in dams can influence the quality of maternal care [66,67], investigating whether maternal care is altered in these dams, is essential in future experiments as it might explain the observed decrease in anxiety- and depressive-like behavior in the offspring.

4.3. Effects of SERT genotype on offspring anxiety- and depressive-like behavior

To investigate whether FLX exposure and ELSD interact with offspring SERT genotype, we assessed behavior of both SERT$^{+/+}$ and SERT$^{+/-}$ offspring. The SLC6A4 gene, which encodes the human SERT, has a known functional variation within the serotonin transporter linked polymorphic region (5-HTTLPR) of the promotor region and is one of the most studied genes in psychiatric disease risk and response to psychiatric medications. Having the short (S) allele of this polymorphism corresponds with lower expression while having the long (L) allele corresponds with higher expression levels of the SERT, which could in turn influence SERT saturation and 5-HT availability during SSRI treatment. In humans, the S-allele is associated with a poorer response to SSRI treatment and more adverse side effects [43]. Since our

SERT$^{+/-}$ offspring also have reduced SERT expression levels, as seen in human S-allele carriers, we expected them to differ in their response to FLX exposure when compared to SERT$^{+/+}$ offspring. Indeed, we found an interaction between SERT genotype and FLX exposure in both the OF and the sucrose preference test, especially in female offspring. Interestingly, the FLX-induced increase in anxiety in the OF was limited to SERT$^{+/+}$ female offspring and not found in SERT$^{+/-}$ female offspring. Similarly, the lowered sucrose preference due to FLX exposure was observed only in SERT$^{+/+}$ female offspring, and not in SERT$^{+/-}$ female offspring. These findings suggest that SERT$^{+/-}$ female offspring are less sensitive to perinatal FLX exposure than SERT$^{+/+}$ female offspring. In mice, postnatal FLX exposure did not interact with SERT genotype to affect anxiety- and depressive-like behavior [40]. As only few other animal studies have investigated the potential interaction between developmental FLX exposure and SERT genotype on adult rodent behavior, further research investigating the potential interaction between early FLX exposure and SERT genotype is warranted as sensitivity to SSRIs in S-allele carriers and SERT$^{+/-}$ rodents may be different during development than in adulthood.

Besides the interaction with FLX exposure, we were also interested in the interaction between SERT offspring genotype and ELSD. The well-known study of Caspi and colleagues showed an interaction between the 5-HTTLPR and adverse life events on enhanced stress susceptibility and risk to develop mental disorders in humans [42]. There has been an ongoing discussion about the replicability of this association both in humans and rodents. Preclinical studies rarely show that SERT$^{+/-}$ rodents have increased vulnerability to develop depressive- or anxiety-like behavior after exposure to early life stress (reviewed in [68]). However, research in SERT$^{+/-}$ mice did show that prenatal stress combined with reduced SERT expression results in offspring with social deficits [69], and clinical data support its potential importance in the human population [70]. Offspring are usually indirectly stressed via the mother using pre- or postnatal stressors resulting in altered stress levels in the dam and/or disturbed mother-pup interactions. In the present study however, we used a pre-gestational stressor early in the life of the dam, which in turn has the potential to similarly affect maternal care and interactions with the offspring. However, we did not check for maternal care and mother-pup interactions, and therefore do not know whether the maternal care was altered. Nonetheless, we found an interaction between SERT offspring genotype and ELSD on anxiety- and depressive-like behavior in male offspring. ELSD-induced reductions in anxiety, as seen by the increased time spent in the center of the OF, were found only in SERT$^{+/-}$ offspring. Likewise, the increased preference for sucrose in offspring from dams exposed to ELSD was only seen in SERT$^{+/-}$ offspring. Our findings suggest that SERT$^{+/-}$ offspring are more sensitive than SERT$^{+/+}$ offspring to the effects of ELSD, corresponding with a few other studies that found an interaction between SERT genotype and early life stress [71,72]. The interaction that was found between SERT offspring genotype and ELSD does implicate a higher sensitivity to ELSD in SERT$^{+/-}$ rats compared to SERT$^{+/+}$ rats, but outcomes appear beneficial, since we observed less anxiety- and depressive-like behavior in the animals as a result of ELSD. Because our stressors are applied early in life, further research is warranted to investigate the effects of stressful life events later in life. Taken together, our data suggest that SERT$^{+/+}$ offspring, particularly females, appear to be more vulnerable to the effects of FLX exposure than SERT$^{+/-}$ offspring. At the same time, SERT$^{+/-}$ offspring, particularly males, appear to be more sensitive to the effects of ELSD than SERT$^{+/+}$ offspring.

4.4. Limitations

The strength of the current study is the use of maternal depression rat model to elucidate the effects of perinatal fluoxetine exposure in offspring, which increases the translational value of the study. However, we would also like to address some limitations. For instance, mother-pup interactions were not observed, since we wanted to disturb litters as little as possible. In hindsight, we cannot exclude whether the FLX-induced effects found in the offspring are a direct effect of FLX exposure, or an indirect effect due to altered maternal care. Although some studies did not observe an effect of FLX treatment on maternal care of the dams [34,36,60,73,74], others did find increased maternal licking and arched-back nursing [35,75,76]. This information in especially important since we found a high mortality rate in the pups and subjective observations imply poor maternal care after FLX treatment [49], which may have influenced some of our results. Furthermore, dams were exposed to stress early in life, and their depression-like phenotype is present during the whole pregnancy as well as the lactating period. This is also the case for the fluoxetine treatment. Our study does not segregate which period within the perinatal period is most critical to the fluoxetine treatment/maternal depression, further research to unravel this is warranted. Lastly, offspring were tested at different ages during behavioral tests starting at 16 weeks in the OF, followed by the age of 26 weeks in the FST and ending with the SPT when 27–30 weeks of age. We cannot exclude that these different timepoints may have biased our results, as in mice it has been reported that the age-increasing variation in emotionality can be mediated by SERT genotype [77]. Although it is not known whether similar effects exist in rats, preferably, animals should have been tested over the behavioral tests without this ‘age gap’ between the OF and FST to exclude such bias. A final limitation of this study is that we did not investigate mechanisms underlying the outcomes reported in this study. However, several studies did find alterations in the brain as a result of perinatal SSRI exposure or maternal stress. To name a few, alterations were found in offspring’s hippocampal 5-HT levels, it’s metabolite 5-hydroxyindolacetic acid, the serotonergic transmission, the serotonin transporter gene expression and the 5-HT turnover (reviewed in [78]). Outside the serotonergic system, also long-term hippocampal plasticity effects of perinatal SSRIs have been reported, including changes in the expression of the brain-derived neurotrophic factor (BDNF [53];) and the epigenetic regulation of the BDNF gene [44,56]. Moreover, developmental fluoxetine was found to reduce cell proliferation in offspring [36]. Regarding synaptic modifications, developmental fluoxetine reversed reduced CA3 spines and synapse density [79]. More hippocampal effects have been reported (e.g. for review, see [78]), and several other brain areas may play a role in the alterations found in the behavioral outcomes of the offspring as well. Similar alterations could have occurred in the offspring exposed to fluoxetine and/or maternal adversity in the rats described in the current study but this assumption is speculative, and the study of underlying mechanisms was beyond the scope of this study. Research is ongoing in a different set of animals where underlying mechanisms in young offspring brains are investigated.

5. Conclusion

In conclusion, our findings indicate that both perinatal FLX treatment and ELSD have, even though small, long-term effects on anxiety and depressive-like behavior in the offspring, depending on sex and genotype. More specific, our results indicate that SERT$^{+/+}$ offspring, especially females, appear to be more sensitive than SERT$^{+/-}$ females to the effects of FLX exposure, while SERT$^{+/-}$ offspring, particularly males, are more sensitive than SERT$^{+/+}$ male offspring to the effects of ELSD on affective behavior. Ultimately, investigating the effects of developmental SERT blockade in offspring with genetic SERT variants may provide useful insights into whether genetic variants of the SERT have a predisposition to develop affective disorders later in life. Furthermore, it allows a better understanding of the potential harmful effects of early-life exposure to SSRIs and maternal stress.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bbr.2020.112657.

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