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# Developmental fluoxetine exposure increases behavioral despair and alters epigenetic regulation of the hippocampal BDNF gene in adult female offspring

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ABSTRACT

A growing number of infants are exposed to selective serotonin reuptake inhibitor (SSRI) medications during the perinatal period. Perinatal exposure to SSRI medications alter neuroplasticity and increase depressive- and anxiety-related behaviors, particularly in male offspring as little work has been done in female offspring to date. The long-term effects of SSRI on development can also differ with previous exposure to prenatal stress, a model of maternal depression. Because of the limited work done on the role of developmental SSRI exposure on neurobehavioral outcomes in female offspring, the aim of the present study was to investigate how developmental fluoxetine exposure affects anxiety and depression-like behavior, as well as the regulation of hippocampal brain-derived neurotrophic factor (BDNF) signaling in the hippocampus of adult female offspring. To do this female Sprague–Dawley rat offspring were exposed to prenatal stress and fluoxetine via the dam, for a total of four groups of female offspring: 1) No Stress + Vehicle, 2) No Stress + Fluoxetine, 3) Prenatal Stress + Vehicle, and 4) Prenatal Stress + Fluoxetine. Primary results show that, in adult female offspring, developmental SSRI exposure significantly increases behavioral despair measures on the forced swim test, decreases hippocampal BDNF exon IV mRNA levels, and increases levels of the repressive histone 3 lysine 27 trimethylated mark at the corresponding promoter. There was also a significant negative correlation between hippocampal BDNF exon IV mRNA levels and immobility in the forced swim test. No effects of prenatal stress or developmental fluoxetine exposure were seen on tests of anxiety-like behavior. This research provides important evidence for the long-term programming effects of early-life exposure to SSRIs on female offspring, particularly with regard to affect–related behaviors and their underlying molecular mechanisms.



## Introduction

Selective serotonin reuptake inhibitor (SSRI) medications are commonly used for the treatment of mood disorders during pregnancy, with up to 10% of pregnant women being prescribed these medications (Cooper et al., 2007; Fleschler and Peskin, 2008; Leung and Kaplan, 2009; Oberlander et al., 2006). SSRIs cross the placental barrier and are present in breast milk; consequently playing a role in fetal and child development (Homberg et al., 2010; Kristensen et al., 1999; Rampono et al., 2004). Chronic SSRI treatment often has few negative effects on the mother, however, recent research suggests long-term effects of pre- and/or-postnatal exposure to SSRIs on neurodevelopment of offspring (Ansorge et al., 2004; Gemmel et al., 2015; Homberg et al., 2010; Pawluski, 2012; Pawluski et al., 2012b; Rayen et al., 2011, 2013, 2014, 2015).

Given that serotonin (5-HT) plays a significant role in brain development, modulating processes such as cell division, differentiation, migration, and synaptogenesis (Azmitia, 2001; Lipton and Kater, 1989), it is not surprising that early exposure to SSRI medications may have a long-term effect on neurodevelopment. Recent clinical studies report

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that neonates exposed to SSRI medications during gestation have an increased risk for complications at birth involving low birth weight, reduced gestational age, and impaired heart rate variability (Moses-Kolko et al., 2005; Oberlander et al., 2009). In addition, clinical reports in infants also show that perinatal exposure to SSRIs may affect neurodevelopment as evidenced by alterations in S100B levels (Pawluski et al., 2009b), impaired serotonergic system functioning (Laine et al., 2003), as well as a disturbed hypothalamic–pituitary-adrenal (HPA) system (Oberlander et al., 2009; Pawluski et al., 2012b).

Although there is limited clinical insight in the long-term outcomes of developmental exposure to SSRI medications on mood disorders in children, preclinical research is showing that perinatal exposure to SSRIs can impact measures of mood, HPA axis physiology and sexual differentiation of the brain and behavior (Noorlander et al., 2008; Pawluski et al., 2012b; Rayen et al., 2011, 2013, 2014). For example, studies in rodent models have shown that there is a well-documented increase in anxiety- and depression-related behavior and modulation of the HPA system, particularly in male rodents developmentally exposed to SSRIs (Ansorge et al., 2004; Ishiwata et al., 2005; Karpova et al., 2009; Noorlander et al., 2008; Pawluski et al., 2012b; Smit-Rigter et al., 2012). In turn, these effects often differ when using a model of maternal depression in such a way that developmental SSRI exposure often reverses the effects of maternal stress on outcomes, particularly in male offspring (Rayen et al., 2011, 2013, 2015).

Although limited, previous research has began to show that developmental SSRI exposure can have very different effects in female offspring. For example, postnatal exposure to fluoxetine increases female sexual receptivity, but decreases male copulatory behavior (Rayen et al., 2013, 2014). Developmental SSRI exposure also reduces juvenile play in male, but not female, offspring, and increases anxiety-like behavior in juvenile male, but not juvenile female, rat offspring (Simpson et al., 2011). With regards to depression-related behavior in female offspring, there have been reports that perinatal SSRI treatment increases depression-related behaviors in juvenile and adult female mice (Lisboa et al. 2007; Popa et al., 2008), while others report that perinatal exposure to fluoxetine decreases anxiety- and depression-like behaviors in adult female mice (McAllister et al., 2012). Thus, further investigation is needed to determine the effects of developmental SSRI exposure on mood-related behavioral outcomes in female offspring.

Developmental SSRI exposure can also have a long term impact on hippocampal plasticity. Recent work has reported that early postnatal fluoxetine exposure significantly increases hippocampal neurogenesis and decreases synaptophysin density in the granule cell layer of the hippocampus in adult female, but not male, offspring (Rayen et al., 2015). In addition, early developmental treatment with fluoxetine can induce long-lasting behavioral impairment, accompanied with changes in hippocampal brain-derived neurotrophic factor (BDNF) mRNA levels in adult male mice (Karpova et al., 2009) and overall changes in global DNA methylation in the hippocampus of male offspring (Toffoli et al., 2014). Furthermore, serotonin transporter (5-HTT) knockout rats, which have elevated levels of 5-HT, display decreased BDNF levels in the hippocampus and frontal cortex, concomitant with epigenetic dysregulation at the BDNF gene – i.e. increased DNA methylation at BDNF promoters IV and VI (Molteni et al., 2010); in these three previous studies female offspring were not investigated.

Alterations of BDNF signaling and changes in hippocampal plasticity have been extensively implicated in the pathophysiology and treatment of mood disorders during adulthood (Castren and Rantamaki, 2010). Similarly, epigenetic changes at the BDNF gene in adulthood have been associated with mood disorders (Boulle et al., 2012). Accumulating evidences from preclinical investigations suggest that negative early-life experiences, including environmental adversity, reduced maternal care, and exposure to toxins, can induce epigenetic reprogramming and increase the susceptibility for the development of emotional dysregulation in adulthood (Roth et al., 2009; Weaver et al., 2004). However, the long-term behavioral effects of early-life SSRI exposure on mental health and the molecular pathways underlying neurobehavioral changes induced by perinatal SSRI exposure are still poorly understood, particularly in female offspring.

The aim of the present study was to investigate the long-term behavioral and molecular effects of developmental exposure to fluoxetine, one of the most popular SSRIs regularly used during pregnancy and the post-partum period (Kiryanova et al., 2013), on development in female offspring. In addition, to better model the clinical situation, a model of maternal stress and depression was used where dams were stressed during gestation (Leuner et al., 2014; O'Mahony et al., 2006; Pawluski, 2012; Smith et al., 2004; van den Hove et al., 2008). Offspring were exposed to fluoxetine during the postnatal period, at a time when neural development in rat offspring is similar to neural development during the 3rd trimester in humans (Romijn et al., 1991). Hence, using this paradigm, we explored the long-term effects of maternal fluoxetine exposure on measures of anxiety and depression-related behaviors as well as the expression of BDNF/TrkB (Neurotrophic tyrosine kinase, receptor, type 2) target genes and epigenetic regulation of BDNF signaling in the hippocampus of adult female offspring.

## Materials and methods

### *Animals and procedures*

All experiments were approved by the Animal Ethics Board of Maastricht University in accordance with Dutch governmental regulations (DEC 2008-157 and 2008–158), the European Communities Council Directive (86/609/EEC). All efforts were made to minimize the pain and stress levels experienced by the animals, to reduce the number of animals used and to utilize alternatives to in vivo techniques, if available.

Twenty-eight adult female Sprague–Dawley rats (250–300 g; Charles River Laboratories, France) and 20 adult male Sprague Dawley rats (Charles River) were used in the present study for breeding. Females were initially housed in pairs in opaque polyurethane bins (48 cm × 27 cm × 20 cm) with ad libitum access to rat chow (Sniff, The Netherlands) and tap water. Rats were kept under standard laboratory conditions in a 12 h:12 h light/dark schedule (lights on at 07:00 h). Dams were bred by putting 1 female and 1 male together in a wire mesh cage. Pregnancy was determined by observation of vaginal plugs (embryonic day 0 — E0). Dams were randomly assigned to stress or control groups on GD14 and restraint of dams in the stress group took place three times daily in transparent plastic cylinders under bright light for 45 min (between 8 and 10 am, 12–2 pm, 4–6 pm) until the end of pregnancy (Rayen et al., 2011; van den Hove et al., 2005; Ward and Weisz, 1984). This time period during pregnancy is when stress can result in postpartum depressive-like behavior in the dam (Leuner et al., 2014; O'Mahony et al., 2006; Smith et al., 2004) and, thus was used as a model of maternal depression.

Litter characteristics such as weight and sex ratio were taken at birth. Litters were culled to 5 males and 5 females on postpartum day 1 (P1; birth day = P0). Dams and litters were randomly assigned to one of two treatment groups: fluoxetine (5 mg/kg/day) or vehicle, for a total of four groups of dams and litters: (1) No stress + Vehicle (NS VEH), (2) No stress + Fluoxetine (NS FLX), (3) Prenatal Stress + Vehicle (PNS VEH), (4) PNS + Fluoxetine (PNS FLX). Weaning of litters occurred on P21, followed by housing of litter groups in clear polyutherane bins (48 cm × 27 cm × 20 cm), four per cage until P30. After P30 females were paired housed with a same sex litter mate in a clear polyutherane bin (48 cm × 27 cm × 20 cm) until sacrifice (P196). Additional work was carried out in adolescent male and female siblings (Rayen et al., 2011) and adult male siblings (Boulle et al., 2015). Adult female offspring were housed in the reverse light cycle (lights off at 7 am) beginning at least 2 weeks prior to behavioral testing. A reverse light cycle was chosen to investigate behaviors during the most active phase on the light cycle