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# Developmental fluoxetine exposure and prenatal stress alter sexual differentiation of the brain and reproductive behavior in male rat offspring

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**Summary** Depression during pregnancy and postpartum is a significant health problem and affects up to 20% of women. While selective serotonin reuptake inhibitor (SSRI) medications are the drug of choice for treatment of maternal depression, the combined effect of maternal depression and perinatal SSRI exposure on offspring development is poorly investigated. Our aim was to determine the role of exposure to fluoxetine during development on sexual behavior and sexually dimorphic brain structures in male offspring using a rodent model of maternal adversity. Sprague-Dawley rat dams were stressed during gestation and were chronically treated throughout lactation with either fluoxetine or vehicle beginning on postnatal day 1. Four groups of offspring were used: (1) Control + Vehicle, (2) Control + Fluoxetine, (3) Prenatal Stress + Vehicle, and (4) Prenatal Stress + Fluoxetine. We show here that developmental fluoxetine treatment decreases the anogenital distance in juvenile male offspring. In adult male offspring, maternal fluoxetine treatment results in a decrease in the number of intromissions, a longer latency to the first intromission, and a longer latency to the first ejaculation. Furthermore, developmental fluoxetine and/or prenatal stress decrease the area of the sexually dimorphic nucleus of the preoptic area (SDN-POA). Prenatal stress, but not exposure to developmental fluoxetine, decreases the number of tyrosine hydroxylase (TH)-positive cells in anteroventral periventricular nucleus (AVPv) and the volume of the posterior bed nucleus of the stria terminalis (pBST) in male offspring. These results provide important evidence for the long-term impact of maternal adversity and maternal fluoxetine use on the development of primary endocrinology systems in juvenile and adult male offspring.


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## 1. Introduction

The early organization of the central nervous system in vertebrates is strongly influenced by the environment. Parental influence during the pre- and post-natal period is by far one of the main environmental inputs affecting the development of brain and behavior (Weinstock, 2001; Talge et al., 2007). As such, any factor affecting the physiology and the behavior of the mother is likely to modulate the developmental output of the offspring. For example, maternal stress, depression and anxiety, can have long-term influences on the physical and mental development of children and can significantly impair cognitive and emotional outcomes, such as general intellectual and language functioning, impulsivity during cognitive tasks, and anxiety (Talge et al., 2007). Similarly in rodent models, maternal stress during gestation can significantly affect offspring outcomes related to cognition and affect-related behaviors (Weinstock, 2001). In addition, gestational stress disrupts the development of the neuroendocrine and reproductive systems in offspring (Maccari et al., 1995).

An estimated 20% of women are affected by depression during pregnancy and the postpartum period (Leung and Kaplan, 2009). Current treatments of maternal depression mainly target the serotoninergic system with selective serotonin reuptake inhibitors (SSRI) being the most frequently prescribed medications for maternal depression both during pregnancy and postpartum (Fleschler and Peskin, 2008). Recent estimates suggest that SSRI use during gestation ranges between 5 and 10% (Oberlander et al., 2006; Cooper et al., 2007). SSRIs cross the placental barrier and are present in breast milk and, therefore, can affect fetal and neonatal development (Kristensen et al., 1999). Serotonin itself is implicated in several developmental processes, including cell division, neural migration, cell differentiation and synaptogenesis (Azmitia, 2001). Thus, altering the availability of serotonin to the neonate, via SSRI exposure, may affect crucial developmental mechanisms. Present clinical and preclinical research has began to show that prenatal SSRI exposure alters serotonin levels (Laine et al., 2003; Weaver et al., 2010) and the hypothalamic-pituitary-adrenal axis (Oberlander et al., 2009; Rayen et al., 2011; Pawluski, 2012; Pawluski et al., 2012a,b). However, it remains unclear how developmental SSRI exposure, and the consequential changes in serotonin, affect key physiological systems related to reproduction in the offspring, such as the hypothalamic-pituitary-gonadal (HPG) system.

Serotonin plays a key role in the development and function of the HPG axis (Ladosky and Gaziri, 1970; Jarzab and Dohler, 1984; Dohler et al., 1991). In addition, serotonin is a major neurotransmitter implicated in the control of sexual behavior during adulthood and fibers originating from the serotoninergic-synthesizing cells in the dorsal raphe strongly innervate sexually dimorphic structures, like the medial preoptic area (MPOA), the bed nucleus of the stria terminalis (BST), and the anteroventral periventricular nucleus (AVPv) (Simerly et al., 1985a; Wilson et al., 1986). This sexual dimorphism is established in the early postnatal period by the action of gonadal steroids and is modulated in part by serotonin Although little work has been done on the effects of developmental exposure to SSRIs on sexual differentiation of the brain and sexual behavior, recent preclinical research has shown that developmental exposure to SSRIs may have a long-term impact on sexual behavior in male offspring. For example, male offspring postnatally exposed to the SSRI citalopram show decreased sexual behavior. However, others did not show significant differences in sexual behavior after prenatal exposure to fluoxetine (Olivier et al., 2011). Although this work points to a potential impact of SSRIs on the development of the HPG axis, it should be noted that these effects were investigated using healthy mothers and offspring, and did not accurately model the clinical situation (Maciag et al., 2006a, 2006c). It remains to be determined how developmental exposure to SSRIs may alter the HPG system, both brain and behavior, when using a model of maternal adversity.

The aim of the present study was to investigate the effect of developmental exposure to the commonly used SSRI, fluoxetine (Prozac®), on the HPG system, using a model of maternal adversity. We focused our attention on the long-term effect of fluoxetine, via maternal exposure, on sexual behavior and the sexually differentiated brain regions, including the AVPv, the BST, and the sexually dimorphic nucleus of the preoptic area SDN-POA in adult male offspring. Insights in the effects of maternal SSRI exposure on the developing HPG system is essential in understanding how these medications affect underlying physiological mechanisms of sexual differentiation.

## 2. Methods

### 2.1. Animals

Forty-five adult female Sprague-Dawley rats (250—300 g; Charles River Laboratories, France) were used as dams in the present study. Twenty-four adult females were used for Experiment 1 (Exp 1) and twenty-one adult females were used for Experiment 2 (Exp 2). Rats were kept under standard laboratory conditions in a 1200 h:1200 h light/dark schedule (lights on at 0700 h), initially housed in pairs in clear polyurethane bins (48 cm × 27 cm × 20 cm) with ad libitum access to rat chow (Sniff) and tap water. All experiments were approved by the Animal Ethics Board of Maastricht University in accordance with Dutch governmental regulations (DEC 2009-158, DEC 2010-150). All efforts were made to minimize the pain and stress levels experienced by the animals.

For breeding, one female was paired with one male in a wire mesh cage until release of a vaginal plug. The day of plug release was considered gestation day (GD) 1. On GD15, dams were randomly assigned to stress or control groups. Dams in the stress group were individually immobilized three times daily for 45 min in transparent plastic cylinders under bright light (13 Watt TL Lamp) (between 0800 h and 1000 h, 1200 h and 1400 h, 1600 h and 1800 h) on GD15—20 and twice on GD21 as previously described (Rayen et al., 2011; Pawluski et al., 2012b). One day after birth (birth day = P0), litters were culled to five males and five females and dams (with offspring) were randomly assigned to one of two treatment groups: fluoxetine (5 mg/kg/day) or vehicle, for a total of four groups of dams: (1) Control + Vehicle (CV), (2) Control + Fluoxetine (CF), (3) Prenatal Stress + Vehicle (PSV), and (4) Prenatal Stress + Fluoxetine (PSF). All litters were

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