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Perinatal exposure to the selective serotonin reuptake inhibitor citalopram alters spatial learning and memory, anxiety, depression, and startle in Sprague-Dawley rats

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ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) block the serotonin (5-HT) reuptake transporter (SERT) and increase synaptic 5-HT. 5-HT is also important in brain development; hence when SSRIs are taken during pregnancy there exists the potential for these drugs to affect CNS ontogeny. Prenatal SSRI exposure has been associated with an increased prevalence of autism spectrum disorder (ASD), and peripheral 5-HT is elevated in some ASD patients. Perinatal SSRI exposure in rodents has been associated with increased depression and anxiety-like behavior, decreased sociability, and impaired learning in the offspring, behaviors often seen in ASD. The present study investigated whether perinatal exposure to citalopram causes persistent neurobehavioral effects. Gravid Sprague-Dawley rats were assigned to two groups and subcutaneously injected twice per day with citalopram (10 mg/kg; Cit) or saline (Sal) 6 h apart on embryonic day (E)6-21, and then drug was given directly to the pups after delivery from postnatal day (P)1-20. Starting on P60, one male/female from each litter was tested in the Cincinnati water maze (CWM) and open-field before and after MK-801. A second pair from each litter was tested in the Morris water maze (MWM) and open-field before and after (+)-amphetamine. A third pair was tested as follows: elevated zero-maze, open-field, marble burying, prepulse inhibition of acoustic startle, social preference, and forced swim. Cit-exposed rats were impaired in the MWM during acquisition and probe, but not during reversal, shift, or cued trials. Cit-exposed rats also showed increased marble burying, decreased time in the center of the open-field, decreased latency to immobility in forced swim, and increased acoustic startle across prepulse intensities with no effects on CWM. The results are consistent with citalopram inducing several ASD-like effects. The findings add to concerns about use of SSRIs during pregnancy. Further research on different classes of antidepressants, dose-effect relationships, timing of exposure periods, and mechanisms for these effects are needed. It is also important to balance the effects described here against the effects of the disorders for which the drugs are given.

1. Introduction

Early brain development can be influenced by factors arising from the environment, gene x environment, and gene x environment x epigenetic interactions. Numerous neurodevelopmental processes, including neurogenesis, migration, cell fate determination, apoptosis, synaptogenesis, arborization, and dendritic pruning are all perturbable processes. Therefore, perinatal exposure to exogenous compounds may, under certain circumstances, alter these processes and change the trajectory of development (Heindel, 2005). Antidepressants (ADs) are monoamine reuptake inhibitors, but the effects on embryonic and fetal brain are not well understood despite their widespread use.

In the United States, depression is common (Kessler, 2012; Kessler et al., 2003; Kessler and Bromet, 2013), and it is prevalent during child-bearing years (Blazer et al., 1994; Kessler et al., 2005; Yonkers et al., 2009). Between 14–23% of women experience depression during pregnancy (Gaynes et al., 2005). Over the past decade, the use of ADs during pregnancy has increased (Alwan et al., 2011; Bakker et al., 2008; Dawson et al., 2016) with 3–4% of preg-

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nant women prescribed these drugs in the United States (Alwan and Friedman 2009; Cooper et al., 2007). Selective serotonin (5-HT) reuptake inhibitors (SSRIs) are the most commonly prescribed ADs and account for up to 20% of ADs prescribed during and after pregnancy (Dayan et al., 2002; Marcus and Heringhausen 2009; Yonkers et al., 2009). Thus, each year, tens of thousands of children are exposed to SSRIs *in utero* (Glover et al., 2015).

Some newborns exposed to SSRIs *in utero* display an adaptation syndrome shortly after birth characterized by erratic heart rate, agitation, vomiting, and altered sleep (Hayes et al., 2012; Levinson-Castiel et al., 2006; Sanz et al., 2005; Zeskind and Stephens 2004). During brain development SSRIs can alter hypothalamic-pituitary-adrenal axis function (Oberlander et al., 2008), sociability, and anxiety in children (Klinger et al., 2011). In addition, recent reports show an increase in autism spectrum disorder (ASD) in children whose mothers took SSRIs before and/or during pregnancy (Boukhris et al., 2016; Croen et al., 2011; Gentile 2015; Gidaya et al., 2014; Harrington et al., 2014; Man et al., 2015; Rai et al., 2013). These studies generally report approximately a doubling of ASD prevalence compared with those not exposed (Boukhris et al., 2016; Croen et al., 2011; Gidaya et al., 2014). ASD prevalence is estimated by the CDC to be about 1 in every 68 children; hence even a doubling would still be only 2 in every 68 children if this hypothesis is correct. Nevertheless, if true, it is a potentially eliminable problem and any eliminable problem should not be ignored. At present, however, the hypothetical connection between prenatal AD exposure and ASD remains unproven (Glover et al., 2015).

In rodents embryonic SSRI exposure increases mortality (Vorhees et al., 1994), decreases growth (Cabrera and Battaglia, 1994), and reduces the 5-HT reuptake transporter (SERT), 5-HT levels, and 5-HT receptors (Cabrera-Vera and Battaglia, 1998; Cabrera and Battaglia, 1994; Hansen and Mikkelsen, 1998). Developmental modification of 5-HT using other types of drugs or gene deletion results in morphological changes in the somatosensory cortex (Lee 2009; Persico et al., 2001; Xu et al., 2004). Moreover, peripheral changes in 5-HT are found in some ASD patients (Chandana et al., 2005; Simpson et al., 2011), as are changes in sensory perception (Kern et al., 2006; Kohl et al., 2014; Leekam et al., 2007). Some prenatal SSRI animal studies report improved spatial learning (Bairy et al., 2007), decreased impulsivity (Lisboa et al., 2007), increased sensitivity to the reinforcing effects of cocaine (Forcelli and Heinrichs, 2008), and increased behavioral despair in the Porsolt forced swim test (Lisboa et al., 2007).

When given neonatally, studies find that SSRI exposure results in alterations in emotion-related behaviors, sociability, and cognition, effects often seen in ASD. Increased depression-like behavior (Hansen et al., 1997), decreased exploration, increased anxiety, (Ansorge et al., 2004), increased anhedonia (Popa et al., 2008), diminished sexual performance (Maciag et al., 2006a), and decreased aggression (Manhaes de Castro et al., 2001) have been seen following neonatal SSRI exposure. More recently, Rodriguez-Porcel et al. (2011) found that postnatal day (P)8-21 SSRI exposure caused altered freezing to a novel auditory stimulus, reduced novel object exploration, and reduced play behavior in juveniles. As adults, these animals showed reduced interactions with unfamiliar conspecifics. Neonatal exposure (P11-20) to citalopram has also been reported to impair egocentric and allocentric learning and memory (Schaefer et al., 2013).

In the mature brain, 5-HT neurons project from the raphe nuclei throughout the brain where they modulate a range of activities such as sensory function (Wei et al., 2010), sleep (Silber and Schmitt, 2010), hunger (Tecott, 2007), sexual behavior (Chan et al., 2011), learning and memory (Buhot et al., 2000; Schmitt et al., 2000), and emotion (Heninger, 2005); these effects being modulatory rather than determinative. Synaptic concentrations of 5-HT are regulated by SERT, the expression of which differs by age and region. SERT is found in all major sensory afferents in monkeys by mid-gestation (see (Homberg et al., 2010)). In mice, SERT mRNA is found in neurons as early as embryonic day (E)11 (Homberg et al., 2010; Narboux-Neme et al., 2008). There is also a placental source during embryogenesis that seeds 5-HT in the raphe nuclei and then disappears (Bonnin et al., 2011). At birth, SERT is transiently expressed in prefrontal and cingulate cortices. It is thought that this expression maintains 5-HT at target receptors to coordinate functional connectivity (reviewed in (Gaspar et al., 2003; Olivier et al., 2011)).

Since SSRIs inhibit SERT, this may change normal serotonergic innervation patterns during development (Olivier et al., 2011). How such effects interact with genetic and/or epigenetic mechanisms, or if they do at all, in relation to ASD is unknown. For example, patients with ASD have hyperserotonemia (Anderson et al., 1990) and alterations in sensory perception (Rogers and Ozonoff, 2005). It has also been suggested that asymmetric development of 5-HT circuitry may contribute to ASD symptoms such as abnormal socialization and motor behaviors (Simpson et al., 2011).

Unfortunately, only limited data exist on the effects of perinatal exposure to SSRIs on offspring. The present study investigated whether pre- and postnatal exposure to citalopram affects behavior in the offspring as adults. The focus was on assessing learning and memory, social preference, anxiety, depression, sensorimotor gating, and drug challenge effects using an NMDA-receptor antagonist (MK-801) and an indirect sympathetic agonist (amphetamine). We did not focus exclusively on ASD-related behaviors, but sought a broad perspective. We exposed gravid female rats from E6-21 to model first and second trimester human equivalent exposure. In order to span third trimester equivalent exposure and avoid issues of drug passage through maternal milk, we exposed pups directly from P1-20. This spanned virtually all stages of brain 5-HT development (Hansson et al., 1998; Homberg et al., 2010; Johnston and Coyle 1980; Lauder and Bloom 1975; Narboux-Neme et al., 2008; Olivier et al., 2011; Zhou et al., 2000). There is no exact point of equivalency between brain development in humans at birth and a particular postnatal day in rats but most comparative analyses place it somewhere between P12-19 (Bayer et al., 1993; Clancy et al., 2001; Clancy et al., 2007), therefore, to ensure coverage we continued treatment through P20. We hypothesized that citalopram exposure would induce enduring effects on offspring behavior as adults.

2. Materials and methods

2.1. Animals

All procedures were approved by the Institutional Animal Care and Use Committee of Cincinnati Children's Research Foundation and conformed to animal use guidelines set forth by the National Institutes of Health. Animals were maintained in an AAALAC International accredited vivarium on a 14–10 h light-dark cycle (lights on at 600 h) with controlled temperature ($19 \pm 1\,^{\circ}C$) and humidity ($50 \pm 10\%$). NIH-07 rodent chow and reverse osmosis filtered, UV sterilized water were freely available. Male and nulliparous female Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC) were acclimated to the vivarium for 1–6 weeks prior to breeding. Females were placed with the males overnight in cages with wire subfloors. The morning a sperm plug was found beneath the subfloor was designated E0; pregnant females were transferred to polycarbonate cages (26×48 cm and 20 cm tall) containing woodchip bedding and a semicircular stainless steel enclosure to provide environmental enrichment (see (Vorhees et al., 2008)). Day of birth was designated P0. On P1, litters were culled using a random number table to 8 pups (4 males and 4 females) where possible. There were cases where litters had unequal sex ratios or fewer than 8 pups. We required at