Hormones and Behavior xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Developmental estrogen exposures and disruptions to maternal behavior and brain: Effects of ethinyl estradiol, a common positive control

Mary C. Catanese[a], Laura N. Vandenberg[a,b,*]

[a] Program in Neuroscience and Behavior, University of Massachusetts – Amherst, USA
[b] Department of Environmental Health Sciences, School of Public Health and Health Sciences, University of Massachusetts – Amherst, USA

ARTICLE INFO

Keywords:
Contraceptive
Endocrine disruptor
Maternal-infant interaction
Open field
Vulnerable period
Organizational effects
Xenoestrogen

ABSTRACT

Due of its structural similarity to the endogenous estrogen 17β-estradiol (E2), the synthetic estrogen 17α-ethinyl estradiol (EE2) is widely used to study the effects of estrogenic substances on sensitive organs at multiple stages of development. Here, we investigated the effects of EE2 on maternal behavior and the maternal brain in females exposed during gestation and the perinatal period. We assessed several components of maternal behavior including nesting behavior and pup retrieval; characterized the expression of estrogen receptor (ER)α in the medial preoptic area (MPOA), a brain region critical for the display of maternal behavior; and measured expression of tyrosine hydroxylase, a marker for dopaminergic cells, in the ventral tegmental area (VTA), a brain region important in maternal motivation. We found that developmental exposure to EE2 induces subtle effects on several aspects of maternal behavior including time building the nest and time spent engaged in self-care. Developmental exposure to EE2 also altered ERα expression in the central MPOA during both early and late lactation and led to significantly reduced tyrosine hydroxylase immunoreactivity in the VTA. Our results demonstrate both dose- and postpartum stage-related effects of developmental exposure to EE2 on behavior and brain that manifest later in adulthood, during the maternal period. These findings provide further evidence for effects of exposure to exogenous estrogenic compounds during the critical periods of fetal and perinatal development.

1. Introduction

The display of maternal behavior and interactions between the mother and her offspring are critical for development and health in rodents and humans alike (Batten et al., 2004; Francis et al., 1999b; Gilbert et al., 2009). Maternal behavior integrates neuroendocrine and physiological systems (Barrett and Fleming, 2011; Bowlby, 1951; Pereira and Ferreira, 2016; Rosenblatt, 1994), involves the interaction of numerous endogenous and environmental factors (Bale et al., 2010; Barrett and Fleming, 2011; Bridges, 2015) and is influenced by estrogen signaling (Pfaff et al., 2011). Furthermore, displaced or dysfunctional maternal behavior can have severe and long lasting neuropsychiatric and medical consequences for children who experience abuse and neglect (Felitti et al., 1998; Gilbert et al., 2009; Gunnar and Fisher, 2006).

The role of endogenous estrogens in maternal behavior, as well as the effects of low doses of exogenous estrogens in the disruption of these behaviors, remains in question. In the laboratory rat, classical studies have shown that at parturition, a decrease in progesterone followed by an increase in 17β-estradiol (E2) is necessary for the onset of maternal behavior, with prolactin, oxytocin and maternal-offspring interactions sustaining its display thereafter (Bridges et al., 1985; Lonstein and Morrell, 2007; Morishige et al., 1973; Numan and Insel Thomas, 2003; Rosenblatt et al., 1988; Shaikh, 1971; Siegel and Rosenblatt, 1978). In laboratory mice, nulliparous females are frequently considered spontaneously maternal, which is taken to suggest a limited role for estrogen in maternal care in this species; pup exposure induces pup retrieval and other maternally relevant behaviors in ovariectomized and aromatase knockout mice (Stolzenberg and Rissman, 2011). However, virgin Swiss mice have also been found to commit infanticide in an intruder test (Parmigiani et al., 1999). Furthermore, a role for hormonal influence in mouse maternal behavior cannot be ruled out because in wild caught mice, ~60% of nulliparous females and ~90% of females during late pregnancy commit infanticide, a response which was found to be mediated by oxytocin. Interestingly, infanticide does not continue after parturition (McCarthy et al., 1986).

Additionally, there is evidence for a role of E2 in the establishment and display of maternal behavior in laboratory mice (Gandelman, 1973;

* Corresponding author at: University of Massachusetts – Amherst, School of Public Health & Health Sciences, Department of Environmental Health Sciences, 171A Goessmann, 686 N. Pleasant Street, Amherst, MA 01003, USA.
E-mail address: lvandenberg@schoolph.umass.edu (L.N. Vandenberg).

http://dx.doi.org/10.1016/j.yhbeh.2017.10.013
Received 31 July 2017; Received in revised form 28 September 2017; Accepted 24 October 2017

Hauser and Gandelman, 1985; Stolzenberg and Rissman, 2011); ovarian hormones have been shown to mediate maternal motivation (Hauser and Gandelman, 1985). Estrogen receptor (ER)α knockout females display poor maternal behavior (Couse et al., 2000; Ogawa et al., 1998) and conditional silencing of ERα in the medial preoptic area (MPOA), a region of the forebrain critical for maternal care, abolishes maternal behavior (Ribeiro et al., 2012). Additionally, pregnant and lactating females have been shown to have increased ER immunoreactivity in the MPOA compared to virgins, with increased receptor density in lactating females (Koch and Ehret, 1989), findings which suggest that ERα is important for the display of mouse maternal behavior.

In non-human primates, circulating concentrations of E2 are similar in abusive and non-abusive mothers both before parturition, when levels are high (500–900 pg/ml), and after parturition, when levels are lower (< 200 pg/ml) (Maestripieri and Megna, 2000a). Yet, rhesus macaque mothers with greater frequencies of abusive behaviors have higher E2:progesterone ratios at the end of pregnancy (Maestripieri and Megna, 2000b), suggesting a role for more complex hormone profiles in the quality of maternal care. In humans, a small study recently revealed polymorphisms in Esr1, the gene encoding ERα, that were associated with negative parenting in mothers (Lahey et al., 2012). These findings are consistent with a role for estrogen in human maternal behavior although the functional consequences of these polymorphisms have not yet been explored.

Endocrine disrupting chemicals (EDCs) are compounds that interfere with hormone signaling (Zoeller et al., 2012) by affecting the synthesis, secretion, transport, binding, action, or elimination of natural hormones (Kavlock et al., 1996). Many of these chemicals have been shown to mimic the actions of estrogen via interactions with ERs (FDA, 2010). Although maternal behavior is not well studied in traditional toxicological evaluations of EDCs, several studies have shown disruptions to maternal care after adult or developmental exposures to a range of EDCs (reviewed in (Catanese et al., 2015; Palanza et al., 2002b; Palanza et al., 2016; Walker and Gore, 2011)).

17α-ethinyl estradiol (EE2) is the active estrogenic component found in oral contraceptives, used by 100 million women worldwide (Petitti, 2003; Pletzer and Kerschbaum, 2014). Due to its affinity for ERα (Anstead et al., 1997; Blair et al., 2000), its structural similarity to the endogenous estrogen E2, and its oral bioavailablity, EE2 has been used to study the effects of estrogens on sensitive organs at multiple stages of development. In fact, EE2 is a common positive control used in studies of other putative EDCs with estrogenic properties (vom Saal et al., 2005).

Two studies of female rats produced conflicting results on the effects of EE2 on maternal behavior after exposures during pregnancy (Arabo et al., 2005; Dugard et al., 2001). In the first study, pup retrieval was assessed; in the latter, pup retrieval and observations on the nest evaluating direct and indirect interactions with offspring (e.g. carrying, licking, moving on the nest) and self-directed behaviors (e.g., eating, drinking) were examined. In both studies, females were injected daily on days 9–14 of pregnancy with 15 μg EE2/kg/day. In addition to the high reproductive toxicity induced by this dose, these two studies produced conflicting effects on maternal behavior even though they were conducted by the same research group and used a similar experimental design. The contradictory results reported made it difficult to draw conclusions on the effects of exogenous estrogens on maternal care and led us to evaluate the effects of EE2 on maternal behaviors. We first assessed maternal behavior and brain in CD-1 mice exposed to low doses during pregnancy and lactation (Catanese and Vandenberg, 2017b). We found that females exposed to 0.01 or 1 μg EE2/kg/day from pregnancy day 9 through lactational day 21 had no significant disruptions to maternal behaviors, although EE2 treatment induced a significant reduction in tyrosine hydroxylase positive cells in the ventral tegmental area (VTA), a brain region important for maternal motivation; these females also spent more time displaying stereotypy behaviors (e.g., repetitive tail retrievals to the nest).

Although these studies suggest that the effects of EE2 on maternal behavior in exposed *adult* female rodents may be subtle or absent, prior evaluations examining neurobehaviors in offspring *developmentally* exposed to EE2 indicate that the pups experience detrimental effects, including those that are detected later in adulthood. In one of the rat studies examining maternal behavior in EE2 exposed mothers, the F1 offspring were found to exhibit behavioral changes including increased spontaneous motor activity, decreased exploration, increased anxiety-like behavior, as well as changes in cognitive processing in adulthood (Dugard et al., 2001). The second study examining maternal behavior in exposed rat dams revealed that EE2 increased anxiety-like and depressive-like behaviors in F1 offspring (Arabo et al., 2005). In a more recent study, female California mice exposed to EE2 during the gestational and perinatal period spent less time nursing and grooming pups, and more time away from the nest compared to controls (Johnson et al., 2015). In another recent study, female Swiss mice were exposed to 0.1 and 1 μg EE2/kg body weight/day from gestational day 10 through postnatal day 40. In addition to effects on reproductive and anxiety-like behaviors, maternal behavior was examined in nulliparous females who spontaneously display maternal behaviors (Derouiche et al., 2015). Interestingly, mice exposed to the lower dose of EE2 had longer latencies to retrieve pups and females from both EE2 groups spent more time in non-pup directed activities. However, these mice were nulliparous, making it difficult to adequately assess effects of developmental EE2 exposures on reproductively associated maternal behaviors.

Here, we examined effects of low doses of EE2 on maternal behaviors in female CD-1 mice exposed in utero and during the perinatal period [the F1 generation, raised from the mothers examined in our previous study (Catanese and Vandenberg, 2017b)]. Based on studies demonstrating that developmental exposures to estrogenic chemicals produce effects with long latency (Heindel and Vandenberg, 2015; Zoeller et al., 2012), we hypothesized that exposure to EE2 during early life would induce deleterious effects that manifest in the context of parenting behavior. In addition to effects on maternal behavior, we examined the effects of developmental EE2 exposures on expression of ERα in the MPOA, a brain region critical for the display of maternal behavior, and dopaminergic neurons in the VTA, a brain region receiving functional input from the MPOA. The VTA is implicated in maternal motivation (Numan, 2007; Numan and Stolzenberg, 2009) and there is evidence that projections from the MPOA to the VTA are responsive to estrogen (Fahrbach et al., 1986; Morrell et al., 1984), indicating that the VTA may be sensitive to endocrine disruption. To our knowledge, this is the first study to examine the effects of developmental low dose EE2 exposures on both the maternal brain and maternal behavior. Because we used the same methods to evaluate the F0 and F1 generation, and also used these methods in studies of another estrogenic EDC (Catanese and Vandenberg, 2017a), the completion of this study allows us to compare two critical periods and the effects of two xenoestrogens.

2. Methods

2.1. Animals

Timed pregnant female CD-1 mice (Charles River Laboratories, Stoneridge, NY), were acclimated for at least two days and individually housed in polysulfone cages (until parturition) with food (ProLab IsoDiet) and tap water (in glass bottles) provided ad libitum. The animals were maintained in temperature (23 ± 2 °C), humidity (40 ± 10%) and light controlled (12 h light, 12 h dark, lights on at 0800 h) conditions at the University of Massachusetts Amherst Central Animal Facility. All experimental procedures were approved by the University of Massachusetts Institutional Animal Care and Use Committee.

From pregnancy day 9 – lactational day 20, F0 dams were provided