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Premarin has opposing effects on spatial learning, neural activation, and serum cytokine levels in middle-aged female rats depending on reproductive history

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ABSTRACT

Menopause is associated with cognitive decline, and hormone therapies (HTs) may improve cognition depending on type and timing of HTs. Previous parity may influence cognition in later life. We investigated how primiparity and long-term ovariectomy influence cognition, neurogenesis, hormones, cytokines, and neuronal activation in middle-aged rats in response to Premarin, an HT. Nulliparous and primiparous rats were sham-ovariectomized or ovariectomized, administered vehicle or Premarin 6 months later, and all rats were trained in the Morris water maze. Premarin improved early spatial learning and memory in nulliparous rats but impaired early learning in primiparous rats. With training, primiparity increased hippocampal neurogenesis, and Premarin decreased immature neurons, regardless of parity. Moreover, Premarin increased serum tumor necrosis factor α and the CXC chemokine ligand 1 (CXCL1) in trained nulliparous, but not primiparous, rats. However, Premarin decreased the expression of the immediate early gene zif268 in the dorsal CA3 region in primiparous rats after training. Thus, primiparity alters how Premarin affects spatial learning, neuronal activation, and serum cytokines. These findings have implications for the treatment of age-associated cognitive decline in women.

Maternal adaptation is the process by which a woman's body has to adapt to allow for a host of physiological changes that must occur to allow for appropriate survival of the fetus. For example, cardiac output and pulmonary function are increased or decreased, respectively, by as much as 50% in gestating women (Grindheim et al., 2012; Savu et al., 2012). Furthermore, the endocrine system is modified, as the placenta releases a variety of hormones in high concentrations including estradiol, progesterone, and corticotropin-releasing hormone (Brett and Baxendale, 2001; Holl et al., 2008). The mother's immune system also undergoes modifications during pregnancy in part to foster tolerance to the forming fetus (Ghaebi et al., 2017). Although it has been widely assumed that many aspects of maternal physiology normalize after the expulsion of the placenta, certain physiological changes outlast the pregnancy and early postpartum and even emerge later in life. For example, as parous women age, mid–luteal phase estradiol levels are decreased compared with nulliparous women (Dorgan et al., 1995). In addition, parity alters the immune profile of aged animals (Barrat et al., 1997). Parity also has lasting consequences on the brain, as long-lasting changes in hippocampal volume and cognition are seen in women and rodents (for review see Roes and Galea, 2015; Hoekzema et al., 2017; Galea et al., 2000; Galea et al., 2014; Barha et al., 2015). Two months after parturition, women exhibit reductions of hippocampal volume, and in other brain regions, and this reduction is still evident 2 years after parturition (Hoekzema et al., 2017). In addition, primiparous rats show reduced hippocampal volume and neurogenesis during lactation and throughout the postpartum period (Galea et al., 2000; Pawluski and Galea, 2007, Leuner et al., 2007; Workman et al., 2015). Thus, studies suggest that reproductive experience has lasting effects on the maternal physiology and hippocampal structure and plasticity.

Neurogenesis in the hippocampus is reduced in the early and late postpartum in primiparous female rats (Galea et al., 2000; Pawluski and Galea, 2007; Leuner et al., 2007; Workman et al., 2015), but curiously in middle age, increased hippocampal neurogenesis is seen in multiparous rats relative to nulliparous rats (Barha et al., 2015). Multiparous rodents have increased levels of

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brain-derived neurotrophic factor, synaptophysin, and spinophilin, as well as more immature neurons, compared with nulliparous rats in middle age (Barha et al., 2015; Cui et al., 2014; Macbeth et al., 2008; Rossetti et al., 2016). Furthermore, in response to estrogens, multiparous middle-aged rats show an enhancement in cell proliferation, whereas nulliparous middle-aged rats do not (Barha and Galea, 2011). In the hippocampus and amygdala, neuropathology (neuritic plaques and neurofibrillary tangles) was positively correlated with parity in older women, but not in men, with most subjects having dementia of probable AD (Beeri et al., 2009), suggesting some neurological consequences of increased parity in women. These findings suggest that increased parity is associated with both increased hippocampal plasticity and pathology well into middle age, long after offspring have been reared.

Previous parity may also influence cognitive ability in middle- and older-aged rodents and perhaps women. Primiparous and multiparous middle-aged rats display better performance compared with nulliparous rats in the dry-land maze (Gatewood et al., 2005; Love et al., 2005), water maze (Barha et al., 2015; Lemaire et al., 2006), and in reversal learning tasks (Gatewood et al., 2005). Importantly, previous reproductive experience may affect various aspects of hippocampal cognition differently. In middle age, multiparous rats have enhanced early acquisition in the spatial working memory version of the Morris water maze, but impaired reference memory acquisition, compared with nulliparous rats (Barha et al., 2015). Studies in women are more equivocal. However, parity is associated with better memory in older women, with factors such as age of first pregnancy, genotype, and amount of parity playing a moderating role (Fox et al., 2013; Karim et al., 2016; Roes and Galea, 2015). Taken together, these studies suggest that previous parity may be associated with improvements in learning and memory in middle age.

Menopause is associated with a decline in circulating estrogens and declines in certain cognitive domains (Weber et al., 2013). Conversely, hormone therapy (HT) in postmenopausal women may improve cognition depending on age at administration and HT composition (Espeland et al., 2017; Hogervorst et al., 2000; Ryan et al., 2008), but other studies do not find a beneficial effect on cognition (Gleason et al., 2015; Henderson et al., 2016). Premarin is a common HT of conjugated equine estrogens (CEEs), composed 50% of estrone sulfate and 0.1% estradiol sulfate. Meta-analyses indicate that fewer studies report positive effects on cognition with Premarin compared with estradiol-based therapies (Hogervorst et al., 2000; Ryan et al., 2008). In addition, the ability of HT to improve cognition in middle age depends on the specific formulation of HT in animals (Baxter et al., 2013; Prakapenka et al., 2018). Furthermore, the effects of HT on cognition in women may be dependent on the timing of when HTs were prescribed (early or late in menopause), with a greater proportion of studies finding detrimental outcomes, or fewer positive outcomes, when HT is initiated 10 or more years after menopause (Espeland et al., 2017; Hogervorst et al., 2000; Ryan et al., 2008), although other studies find no such effect (Henderson et al., 2016). Theses inconsistencies in the literature may have to do with reproductive history, which is often not included in the analyses, and in the present study, we were interested in whether Premarin's effects on cognition were dependent on past reproductive history.

In a number of studies, a "critical window" exists during which estradiol administration early, but not late, after menopause or ovariectomy improves hippocampus-dependent cognition in middle age (Daniel and Bohacek, 2010; Gibbs, 2010; Vedder et al., 2014; Walf et al., 2009). However, the duration of ovariectomy itself, without HT, can influence cognition and may depend on parity, with long-term (6 months) ovariectomy improving spatial memory in nulliparous rats (Bimonte-Nelson et al., 2003) or showing no cognitive benefit in multiparous rats (Walf et al., 2009), suggesting that parity modulates the ovariectomy-induced effects on cognition in later life. Thus, in the present study, we investigated the effects of long-term ovariectomy on cognition with previous parity.

Reproductive experience can alter the cognitive and neuroplastic effects of estrogens. Estrone and estradiol administration upregulated cell proliferation in the hippocampus of middle-aged multiparous but not nulliparous rats (Barha and Galea, 2011), suggesting that parity preserves the sensitivity of the hippocampus to estradiol and estrone later in life. Furthermore, ovariectomy in middle age has opposing effects on memory in nulliparous versus multiparous rats, improving spatial memory retrieval in nulliparous rats but impairing it in multiparous rats (Barha et al., 2015). Thus, reproductive experience alters hippocampus-dependent memory and neuroplastic response to estrogens in middle age.

Few studies have examined the potential mechanisms of long-lasting alterations in learning and neuroplasticity across aging that depend on parity. Given that steroids and peptide hormones are highjacked during pregnancy, it is likely that there may be long-term impacts to these endocrine systems (Barha and Galea, 2017). Although it is not known what mechanisms contribute to these long-term changes, current knowledge suggests a role of estrogens, adrenal hormones, and inflammatory signaling to drive long-term changes with parity. Studies in women point to reductions in estrogens across the menstrual cycle after parity (Barrett et al., 2014), and estrogens are related to both neuroplasticity and cognition (for review see: Duarte-Guterman et al., 2015). Cortisol and corticosterone can influence hippocampus-dependent cognition and neuroplasticity (for review see Wingenfeld and Wolf, 2014), but studies are mixed as to whether long-lasting alterations to the hypothalamic pituitary adrenal axis are seen with parity (Federenko et al., 2006; Lankarani-Fard et al., 2001; Tu et al., 2006). Furthermore, immune signaling is challenged with the placenta and fetus (Bonney, 2016), and long-lasting changes in immune signaling may be present (Asztalos et al., 2010; Clendenen et al., 2011), which also influence cognition (Donzis and Tronson, 2014). Thus, the mechanisms by which parity can alter the aging trajectory are unclear but may involve changes in inflammatory signatures (Barrat et al., 1997; Cramer and Vitonis, 2017), levels of estrogens (Dorgan et al., 1995; Bridges and Byrnes, 2006; Barrett et al., 2014), and hypothalamic pituitary adrenal axis changes with parity. Given these findings, levels of estrogens, adrenal mass, and inflammatory signatures were examined in the present study.

The aim of the present study was to determine the effects of primiparity, Premarin, and long-term ovariectomy on spatial memory, and how the experimental manipulations affect hippocampal neurogenesis and neuronal activation in behaviorally trained middleaged rats. We also examined possible mediating factors such as adrenal mass, serum levels of cytokines, and estrogens. We hypothesized that Premarin treatment in middle-aged rats would affect spatial reference and reversal learning (a measure of cognitive flexibility), neurogenesis, and neuron activation in the hippocampus in a manner that is dependent on reproductive experience and hormone status.

1. Method

1.1. Animals

Ninety-seven female and 16 male Sprague-Dawley rats (Charles River, Quebec) were 2 months old at arrival at the University of British Columbia. Rats were housed in opaque polyurethane bins (24 × 16 × 46 cm) with aspen chip bedding and were given standard laboratory chow (Harlan, Canada) and tap water *ad libitum*. Rats were maintained under a 12-:12-h light/dark cycle (lights on at