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Higher levels of estradiol replacement correlate with better spatial memory in surgically menopausal young and middle-aged rats

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ABSTRACT

The current study investigated whether, for spatial reference memory, age impacts (1) sensitivity to surgical ovarian hormone loss (Ovx), (2) response to estradiol therapy (ET), and (3) the relation between circulating estradiol levels and memory scores in ovary-intact sham and Ovx plus ET rats. Young, middle-aged and aged Fischer-344 rats received sham, Ovx or Ovx plus ET treatments, and were then tested on the Morris maze. After the last test trial, a probe trial was given whereby the platform was removed. Circulating estradiol levels were then determined and correlated with performance. In Study 1, Ovx facilitated learning on day one, but impaired performance after day one, in young rats. Ovx did not influence performance in middle-aged rats. In young and middle-aged Ovx rats, ET enhanced performance with higher exogenous estradiol levels correlating with better performance during testing and the probe trial. There was no relationship between endogenous estradiol levels and performance in sham young or middle-aged rats. Study 2 showed that, like middle-aged rats, aged rats were not impacted by Ovx. Further, for aged Ovx rats, the ET regimen that was beneficial at earlier ages was no longer effective during test trials, and had only minor benefits for platform localization as assessed by the probe trial. Collectively, the findings suggest that the effects of Ovx as well as responsivity to the currently utilized ET regimen changes with age. Further, there appears to be a distinction between sensitivity to Ovx and responsiveness to ET after Ovx for spatial reference memory performance.

1. Introduction

There is abundant clinical and basic science evidence that estrogens impact cognitive function (Dohanich, 2002). Since the first controlled clinical evaluation showing that estrogen injections given to 75-year-old women enhanced memory (Caldwell & Watson, 1952), there have been numerous studies showing cognitive decline after ovarian hormone loss, and enhancement after estrogen replacement, in menopausal women (Sherwin, 2006). However, several newer clinical studies, including the large, placebo-controlled, multi-center Women's Health Initiative Memory Study, have indicated that certain regimens of hormone treatment, for example, use of conjugated equine estrogens with or without medroxyprogesterone, can have null or detrimental effects on cognition and dementia risk in women (Mulnard et al., 2000; Rapp, Morrison, & Roberts, 2003; Shumaker et al., 2003, 2004; Wang et al., 2000).

The majority of the animal studies evaluating the activational effects of estrogens on learning and memory have been performed in young rodents (Bimonte & Denenberg, 1999; Daniel, Fader, Spencer, & Dohanich, 1997; Daniel, Roberts, & Dohanich, 1999; Dohanich, Fader, & Javorsky, 1994; Galea et al., 2001; Holmes, Wide, & Galea, 2002; Luine, Jacome, & Maclusky, 2003; Luine, Richards, Wu, & Beck, 1998; Marriott & Korol, 2003; Packard & Teather, 1997; Sandstrom & Williams, 2001; Singh, Meyer, Millard, & Simpkins, 1994). While there has been a recent increase in the number of studies evaluating estrogen effects in middle-aged or older female rodents, most using the most potent estrogen, estradiol (Bimonte-Nelson, Francis, Umphlet, & Granholm, 2006; Foster, Sharrow, Kumar, & Masse, 2003; Frick, Fernandez, & Bulinski, 2002; Gibbs, 2000; Luine & Rodriguez, 1994; Markham, Pych, & Juraska, 2002; Markowska & Savonenko, 2002a; Savonenko & Markowska, 2003; Ziegler & Gallagher, 2005), there is only limited work comparing estrogen replacement effects in ovariectomized (Ovx) animals at different ages within the same study (Foster et al., 2003). Hence, it is still a question whether age changes responsiveness to ovarian hormone replacement. This is a clinically important question, as some menopausal women begin hormone

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therapy at a younger age, while some start later in life. Women in the Women's Health Initiative Memory Study were 65 years of age and over, which may have played a critical role in the lack of efficacy of estrogen treatment since age may impact responsiveness (see Craig, Maki, & Murphy, 2005).

Animal work that has been done testing estradiol replacement at multiple ages showed that administered estradiol dose impacted spatial memory retention, and that these effects interacted with age in Ovx rodents (Foster et al., 2003). We and others have also noted divergent cognitive effects depending on administered estradiol dose in mice and rats (Bimonte & Denenberg, 1999; Bimonte-Nelson et al., 2006; El-Bakri et al., 2004; Frick et al., 2002; Gresack & Frick, 2004; Holmes et al., 2002; Packard & Teather, 1997; Rissanen, Puolivali, van Groen, & Riekkinen, 1999). In these studies, however, serum hormone levels were estimated based on prior studies or manufacturer reports (Bimonte & Denenberg, 1999; Bimonte-Nelson et al., 2006; El-Bakri et al., 2004; Frick et al., 2002; Gresack & Frick, 2004; Holmes et al., 2002; Packard & Teather, 1997; Rissanen et al., 1999), or circulating estradiol levels were determined but statistical correlations with performance were not reported (Foster et al., 2003). In fact, in no animal study testing estradiol replacement have serum levels within individual animals been correlated with memory scores. There may be a positive correlation between circulating estradiol level and memory, as seen with endogenous levels in healthy menopausal women (Phillips & Sherwin, 1992; Wolf & Kirschbaum, 2002), and after exogenous estradiol administration to postmenopausal women with probable mild to moderate Alzheimer's disease (Asthana et al., 1999). Alternatively, the relation between serum estradiol level and memory could hold to an inverted U-shaped function, whereby very low or very high values result in the poorest cognitive function. Many biological systems fit this quadratic function (Bimonte, Hunter, Nelson, & Granholm, 2002), and low, but not high, estradiol levels have been associated with better cognitive performance in older women (Barrett-Connor & Goodman-Gruen, 1999). Hence, while some studies suggest that estradiol treatment influences cognition in women and the rodent model, the relation between circulating level of estradiol and memory performance is unclear.

We previously showed that Ovx did not influence spatial reference memory performance in middle-aged female rats (Bimonte-Nelson et al., 2006). Yet, in this age group estradiol replacement resulted in marked enhancements in spatial reference memory maze performance (Bimonte-Nelson et al., 2006). These data suggest that (1) naturally circulating, endogenous estradiol in an ovary-intact individual may relate to cognition in a different way than exogenously administered estradiol due to replacement after Ovx, and (2) sensitivity to ovarian hormone loss does not predict sensitivity to estradiol replacement for spatial reference memory. We and others have shown, in young rats, working memory deficiencies after Ovx and enhancements after estradiol treatment (Bimonte & Denenberg, 1999; Daniel et al., 1997; Daniel et al., 1999; Holmes et al., 2002; Sandstrom & Williams, 2001). Additionally, in middle-aged rats no reference memory deficiencies were seen after Ovx, but benefits of estradiol replacement were observed (Bimonte-Nelson et al., 2006). While suggestive of differences in response to ovarian hormone loss and replacement depending on age, these separate studies could have been due to memory type and not age.

The current studies examined the spatial reference memory effects of Ovx and estradiol replacement in young, middle-aged and aged rats. We and others have found significant variability in circulating estradiol levels after treatment with the same manufacture-labeled dose of estradiol pellets from Innovative Research of America (unpublished observations; Diel, Laudenbach-Leschowsky, Friedel, Voss, & Roussel, 2005). Here we capitalized on this range of serum estradiol levels to investigate relation with memory scores. Circulating estradiol levels were determined in all animals with the intention of correlating these values with maze performance. We performed correlation analyses in animals that were ovary-intact to determine associations between *endogenous* estradiol levels and performance, and within animals that had received Ovx plus estradiol replacement to determine relations between *exogenous* estradiol levels and performance. The relation between circulating estradiol and cognition may be affected by whether the estradiol is endogenous (via an intact ovary) or exogenous (experimentally administered) due to presence of progesterone in ovary-intact animals (Bimonte-Nelson, Nelson, & Granholm, 2004b; Bimonte-Nelson, Singleton, Williams, & Granholm, 2004a; Bimonte-Nelson et al., 2006; Johansson, Birzniece, Lindblad, Olsson, & Backstrom, 2002; Nilsen & Brinton, 2002a, 2002b; Woolley & McEwen, 1992), as well as the cyclicity of estradiol exposure (e.g. cyclic from the ovary vs. a tonic regimen of replacement; Bimonte-Nelson et al., 2004a, 2006; Gibbs, 2000; Johansson et al., 2002; Markowska & Savonenko, 2002a; Nilsen & Brinton, 2002a, 2002b; Woolley & McEwen, 1992).

2. Materials and methods

2.1. Subjects and treatment procedures

For Study 1, subjects were 34 young (4 months old at test) and 33 middle-aged (16 months old at test) Fischer-344 female rats born and raised at the National Institute on Aging at Harlan Laboratories (Indianapolis, IN). After arrival, animals were pair housed, had exposure to food and water ad lib, and were maintained on a 12-h light/dark cycle. All procedures were approved by IACUC and adhered to NIH standards. Each young and middle-aged group contained the following treatment groups: ovary-intact sham (Sham), Ovx with no hormone treatment (Ovx), Ovx plus a 0.25 mg/60 day release estradiol pellet and Ovx plus a 0.50 mg/60 day release estradiol pellet. Thus, there were a total of eight groups: Young-Sham ($n = 8$), Young-Ovx ($n = 9$), Young-Ovx + 0.25 estradiol pellet ($n = 8$), Young Ovx + 0.50 estradiol pellet ($n = 9$), Middle-aged-Sham ($n = 8$), Middle-aged-Ovx ($n = 9$), Middle-aged Ovx + 0.25 estradiol pellet ($n = 8$), and Middle-aged Ovx + 0.50 estradiol pellet ($n = 8$). Ovx surgery was performed two months before testing at 2 and 14 months of age for young and middle-aged groups, respectively. Rats were anesthetized with an intraperitoneal injection of 70 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, IA, USA)/and 6 mg/kg xylazine (Lloyd Laboratories, Shenandoah, IA, USA). For Ovx, bilateral dorsolateral incisions were made in the skin and peritoneum, and the ovaries and tips of uterine horns were ligatured and removed. The muscle was then sutured and the skin stapled. Sham surgery consisted of skin incision and staple in the same fashion.

Estradiol replacement via pellets (Innovative Research of America, Sarasota, Florida) was administered one month after Ovx, one month before testing ensued, at 3 months of age for young rats, and 15 months of age for middle-aged rats. Since pellets released hormone for 60 days, animals with the pellets received estradiol for the entire study, including during behavioral testing and through sacrifice. Under Ketamine/Xylazine anesthesia, a small incision was made in the scruff of the neck, and a subcutaneous pocket was created. For animals receiving estradiol, one pellet of the appropriate dose was inserted and the skin stapled. The groups not receiving estradiol replacement (Sham and Ovx groups) received a sham pellet surgery, which included identical procedures except the pocket was left empty.

For Study 2, subjects were 6 young (4 months old at test) and 21 aged (24 months old at test) Fischer-344 female rats born and raised at the aging colony of the National Institute on Aging at Harlan Laboratories (Indianapolis, IN). There were a total of four groups in Study 2: Young-Sham ($n = 6$), Aged-Sham ($n = 6$), Aged-Ovx ($n = 7$), and Aged-Ovx-E ($n = 7$). Just one type of estradiol pellet was chosen for this study (0.25 mg pellet) since the variability and mean levels were comparable between the 0.25 and 0.50 doses in Study 1. The timeframes and surgical procedures of Study 2 were identical to those of Study 1.

2.2. Assessment of serum hormone levels

For both studies, after behavioral testing, rats were anesthetized with Halothane anesthesia and decapitated. Blood was collected from the trunk (Vacutainer 367986, Becton Dickinson and Company, Franklin Lakes, NJ), was allowed to clot at 4 °C, and serum was collected after centrifugation (3220*g*, 20 min). Serum was stored at −20 °C until estradiol assays were performed by the Core Endocrinology Laboratory at Pennsylvania State University College of Medicine using a kit from Diagnostic Products Corporation, Los Angeles, CA (Coat-A-Count estradiol kit, product number TKE21). Estradiol was determined in serum by a solid-phase radioimmunoassay based on estradiol-specific antibodies that are immobilized to the wall of polypropylene tubes and ^{125}I-labeled estradiol as the tracer following extraction with diethyl ether. Serum (2.4 ml) were extracted and the ether portion