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CONTINUOUSLY DELIVERED OVARIAN STEROIDS DO NOT ALTER DENDRITIC SPINE DENSITY OR MORPHOLOGY IN MACAQUE DORSOLATERAL PREFRONTAL CORTICAL NEURONS

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Abstract—Aged ovariectomized (OVX) female monkeys, a model for menopause in humans, show a decline in spine density in the dorsolateral prefrontal cortex (dlPFC) and diminished performance in cognitive tasks requiring this brain region. Previous studies in our laboratory have shown that long-term cyclic treatment with 17β-estradiol (E) produces an increase in spine density and in the proportion of thinner spines in layer III pyramidal neurons in the dlPFC of both young and aged OVX rhesus monkeys. Here we used 3D reconstruction of Lucifer yellow-loaded neurons to investigate whether clinically relevant schedules of hormone therapy would produce similar changes in prefrontal cortical neuronal morphology as long-term cyclic E treatment in young female monkeys. We found that continuously delivered E, with or without a cyclic progesterone treatment, did not alter spine density or morphology in the dlPFC of young adult OVX rhesus monkeys. We also found that the increased density of thinner spines evident in the dlPFC 24 h after E administration in the context of long-term cyclic E therapy is no longer detectable 20 days after E treatment. When compared with the results of our previously published investigations, our results suggest that cyclic fluctuations in serum E levels may cause corresponding fluctuations in the density of thin spines in the dlPFC. By contrast, continuous administration of E does not support sustained increases in thin spine density. Physiological fluctuations in E concentration may be necessary to maintain the morphological sensitivity of the dlPFC to E.

Key words: prefrontal cortex, estrogen, progesterone, aging, primate, hormone replacement therapy.

INTRODUCTION

Levels of estrogens drop off precipitously in women as they go through menopause, and age-related cognitive decline can begin in the decade following the typical age of menopause. This decline is particularly apparent on tasks that rely on the dorsolateral prefrontal cortex (dlPFC), such as those that emphasize working memory and cognitive flexibility (Drogos et al., 2013; Weber et al., 2013). The interaction between this loss of estrogens and the risk of decline in cognitive function is not well understood, and studies designed to explore the cognitive benefits of hormone therapy in women have yielded conflicting results. Although some laboratory studies and randomized clinical trials have found that initiation of hormone replacement therapy (HRT) during perimenopause or soon after the menopausal transition can improve cognitive function (Carlson et al., 2001; Keenan et al., 2001) and reduce a woman's risk of developing cognitive impairment or dementia later in life (Kimura, 1995; Matthews et al., 1999; Carlson et al., 2001; Zandi et al., 2002; Bagger et al., 2005; Henderson et al., 2005; Greendale et al., 2009), others have found that initiation of HRT more than a few years after menopause is associated with an unchanged or increased risk of dementia and age-associated cognitive decline (Matthews et al., 1999; Shumaker et al., 2003, 2004; Henderson et al., 2005; MacLennan et al., 2006), and several randomized clinical trials have found equivocal or negative effects of HRT on cognitive function, even when initiated soon after menopause (reviewed in Maki and Sundermann, 2009).

One factor that may contribute to these discrepancies is the fact that menopausal women are most commonly prescribed a continuous regimen of one or more estrogens with or without a progestin. There is evidence from rodent studies that treatments consisting of a continuous dose of 17β-estradiol (E), the predominant active estrogen in young women (Stricker et al., 2006), may be less effective in enhancing cognitive function than are treatments that provide E on a cyclical schedule, i.e., one dose of E per cycle length (Markowska and Savonenko, 2002). We have previously reported that cyclical E treatment with one dose of E every 21 days for 2–3 years will improve the performance of aged ovariectomized (OVX) female rhesus monkeys on dlPFC-dependent tasks (Rapp et al., 2003) and that the same schedule of cyclic E

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Abbreviations: ANOVA, analysis of variance; CA1, cornu ammonis area 1; DAPI, 4,6-diamidino-2-phenylindole; dlPFC, dorsolateral prefrontal cortex; E, 17β-estradiol; HRT, hormone replacement therapy; NHP, nonhuman primate; OVX, ovariectomized; P, progesterone; PBS, phosphate-buffered saline; SEM, standard error of the mean.

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treatment given for 3 weeks or for 2–3 years increases the density of dendritic spines on dlPFC pyramidal neurons in both young and aged animals (Tang et al., 2004; Hao et al., 2006, 2007). Higher levels of spine and synapse density in the dlPFC have been found to correlate with preservation of dlPFC function in aging rhesus monkeys (Peters et al., 1998; Dumitriu et al., 2010).

In order to determine whether treatment schedule affects the ability of E to alter dlPFC neuronal morphology, the present study examined whether Continuous E therapy, with or without progesterone (P), is effective at increasing thin spine density in the dlPFC of young OVX monkeys. We also examined whether thin spine density in the dlPFC falls during the interval between injections in OVX monkeys receiving cyclical E therapy. We found that Continuous E treatment fails to trigger an increase in spine density, and that the presence or absence of a Cyclic P treatment component does not affect this result. Additionally, we found that spine density in cyclic E-treated animals does decrease between E treatments when circulating E levels are low, and is indistinguishable from that of vehicle-treated animals by 20 days post-E administration.

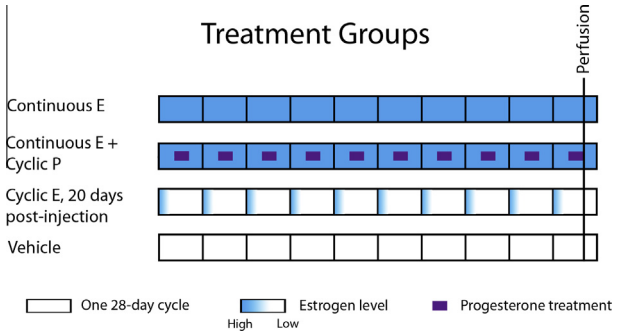

Fig. 1. Hormone therapy treatment groups. All hormone therapy was delivered using a calendar of ten 28-day treatment periods, each representing one menstrual "cycle." The Continuous E group received a single, steady dose of estradiol through subcutaneously implanted Silastic capsules for the duration of the study. The Continuous E + Cyclic P group received the same continuous estradiol treatment, with the addition of an oral dose of progesterone taken daily on days 10–19 of each 28-day treatment cycle. The Cyclic E group received an injection of estradiol intramuscularly on the first day of each 28-day treatment cycle. The vehicle group received an injection of oil vehicle on the first day of each 28-day treatment cycle. All animals were perfused on day 20 of the 10th treatment cycle.

EXPERIMENTAL PROCEDURES

Animals

Twenty young adult female rhesus monkeys (Macaca mulatta; age range, 7.6–14.7 years old; mean age ± standard error of the mean (SEM), 10.1 years ± 6.8 months) were used in this study. Animals were singly housed in colonies of 40 individuals under conditions identical to those used in previous studies (Rapp et al., 2003; Hao et al., 2006, 2007), and water and monkey chow were provided in excess of nutritional needs. All monkeys received bilateral OVX prior to the initiation of hormone therapy as in (Rapp et al., 2003). Briefly, animals were sedated with ketamine (10 mg/kg) and atropine (0.04 mg/kg), intubated, and placed on isoflurane anesthesia. After the ovaries were removed, the abdominal wall was closed in layers, and animals were observed until responsive. Oxymorphone (1.5 mg/kg) was administered three times/day for 2 days for postoperative analgesia. The mean duration between OVX and the beginning of treatment was 51 days (range 42–57 days). All experiments were conducted in compliance with the National Institutes of Health Guidelines for the Care and Use of Experimental Animals approved by the Institutional Animal Care and Use Committee at the University of California, Davis and the Mount Sinai School of Medicine.

Hormone treatment

Five animals were randomly assigned to each of four treatment groups (Fig. 1), which were as follows:

Continuous E

- Animals in this group received a continuous regimen of unopposed E. Each animal was sedated with ketamine (10 mg/kg) and medetomidine (0.2–0.4 mg/kg) and subcutaneously implanted with two 6.5-cm lengths of Silastic tubing (inner diameter, 3.4 mm; outer diameter, 6.4 mm; Dow Corning, Midland, MI, USA), each containing a 5-cm column of packed crystalline E (Sigma–Aldritch, St. Louis, MO, USA). These were designed to produce a target circulating estradiol level of approximately 80 pg/mL, corresponding to the mean serum E level in cycling rhesus monkeys during the luteal and mid-follicular phases of the menstrual cycle (Walker et al., 1983; Monfort et al., 1987).

Continuous E + Cyclic P

- Animals in this group received a continuous regimen of E with a cyclical regimen of P. Each animal was implanted with E-filled Silastic tubing in a manner identical to the animals in the Continuous E group. Each animal additionally received a regimen of one 100-mg capsule of P administered orally once per day on days 11–20 of a 28-day treatment cycle. This regimen was designed to mimic the broad P peak in the luteal phase and near-absence of circulating P in the follicular phase of the natural rhesus menstrual cycle (Walker et al., 1983; Monfort et al., 1987).

Cyclic E, cycle day 20

- Each animal received a single injection of estradiol cypionate (100 μg in 1 ml of sterile peanut oil, IM; Andersham Pharmacia, Peapack, NJ, USA) on day 1 of each 28-day cycle. This is the same dose of the same hormone used in previous studies, though on a 28-day rather than a 21-day cycle (Rapp et al., 2003; Hao et al., 2006, 2007), and has been shown to produce a peak in circulating E levels averaging nearly 300 pg/ml within 9 h, which falls to zero over the next several days (Rapp et al., 2003). This regimen is designed to mimic the preovulatory surge in serum E levels present in cycling monkeys, which rise to a