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NO EFFECT OF DIFFERENT ESTROGEN RECEPTOR LIGANDS ON COGNITION IN ADULT FEMALE MONKEYS

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Abstract

Many studies in women and animal models suggest that estrogens affect cognitive function. Yet, the mechanisms by which estrogens may impact cognition remain unclear. The goal of the present study was to assess the effects of different estrogen receptor (ER) ligands on cognitive function in adult ovariectomized female rhesus monkeys. The monkeys were tested for 6 weeks on a battery of memory and attentional tasks administered on a touchscreen: the object, face, and spatial versions of the Delayed Recognition Span Test (DRST) and a Visual Search task. Following a 2-week baseline period with oil vehicle treatment, monkeys were randomly assigned to one of 3 treatment groups: estradiol benzoate (EB), selective ER β agonist (diarylpropionitrile DPN) or selective ER modulator tamoxifen (TAM). In each treatment group, monkeys received oil vehicle for 2 weeks and the drug for 2 weeks, in a cross-over design. After a 4-week washout, a subset of monkeys was re-tested on the battery when treated with a selective ER α agonist (propyl-pyrazole-triol, PPT) or oil vehicle.

Overall, drug treatments had no or negligible effects on cognitive performance. These results support the contention that exogenous estrogens and selective estrogen receptor modulators (SERMS) do not significantly affect cognition in young adult female macaques. Additional studies are needed to determine whether the cognitive effects of estrogens in monkeys of more advanced age are mediated by ER β , ER α or complex interactions between the two receptors.

Keywords

memory; attention; selective estrogen receptor modulator; DPN; PPT; macaque; hormonal replacement therapy

INTRODUCTION

Estrogens have multiple effects on the brain and impact selective aspects of cognitive function in animal models and humans [1]. For example, estradiol replacement typically affect tasks of verbal fluency, verbal memory and working memory in menopausal women [1]. In ovariectomized (OVX) rodents, several aspects of learning and memory, including spatial

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memory, working memory and learning strategy are also positively or negatively influenced by estradiol treatment [2,3]. In nonhuman primates, the effects of estradiol on cognition have been most consistently observed for tasks of spatial working memory and visuospatial attention (see for reviews [4-6]). The effects of estrogens on cognition are not uniform, however, and depend on a variety of factors that remain to be fully understood. Among these factors, the cognitive domain under investigation, the age of the individuals, the duration of estrogen deprivation, the type and dose of estrogen, the timing and route of administration, are likely to be critical in influencing the nature of estrogen effects on cognition [1]. In addition, at least two estrogen receptor subtypes coexist in the brain, ER α and ER β , which each have a specific regional distribution (in rats: [7]; in monkeys: [8,9]; in humans: [10]) and most likely hold distinct biological functions [11,12]. Indeed, accumulating evidence suggests that ER α is critical in regulating female sexual behavior [13], while ER β is more involved in the regulation of hippocampal synaptic plasticity [14], hippocampal LTP [15] and cerebellar plasticity [16]. Thus, predominant activation of one or the other receptor may elicit different cognitive responses.

One way to infer the potential roles of ER α and ER β in mediating estrogen effects on cognition is to study memory in ER knockout mice. Such studies have provided evidence for a role of ER β in hippocampal-dependent memory tasks, including in the acquisition of spatial reference memory [17], and performance on delayed non-matching-to-position [14], object recognition and object placement tasks [18]. Importantly, ER β may also mediate the anxiolytic properties of estrogens, as ER β knockouts, but not ER α knockouts, show increased anxiety compared to wild-type mice [19].

Another approach to evaluate the roles of ER α and ER β in mediating the effects of estrogens on cognition is to use selective ER α and ER β agonists and antagonists. Diarylpropionitrile (DPN) is an ER β agonist with 70 times greater activity at ER β than ER α [20], and propylpyrazole-triol (PPT) is a ER α agonist, which has a 410-fold selectivity for ER α over ER β [21]. The few studies that have used these compounds to examine the role of ER subtypes on memory in mice and rats have confirmed the key role of ER β in hippocampal-dependent memory [14,15,22]. In addition, treatment with ER β -selective agonists is associated with reduced anxiety in rodents [23-26]. Whether similar effects may hold for primates is currently unknown. Among ER antagonists, the selective ER modulator Tamoxifen (TAM) is often used. TAM can have both ER agonistic and antagonistic properties depending on the tissue [27, 28], but is considered to have ER antagonist effects in the brain [28-30]. Yet, data on TAM effects in the CNS remain unclear. Indeed, several studies have shown that TAM administered alone in OVX animals (in the absence of estradiol) affects brain function. For example, TAM acted as an ER α agonist in the gonadotrope of OVX rats [31]; in OVX macaques, TAM administered alone acted as an anxiogenic agent [32], and diminished ACTH and cortisol response to exogenous CRH [33]. Thus, TAM does not act as a pure ER antagonist in the brain. TAM is used as treatment in combination with chemotherapy in women with breast cancer and has been reported to impair cognitive function in several [34] [35] [36] but not all studies in these patients [37].

The objectives of the present study were to clarify the roles of ER α and ER β in mediating the effects of estrogens on cognition in female nonhuman primates. We tested OVX female rhesus monkeys on a battery of cognitive tasks when treated with estradiol benzoate (EB), DPN, TAM, PPT or oil vehicle. Our predictions, based on the literature, were that (1) EB would modulate performance on selective tasks of the battery; (2) TAM would impair cognitive performance (3) DPN, but not PPT, would mimic the effects of EB on cognition.

METHODS

Subjects

Twenty-one adult ovariectomized (OVX) female rhesus monkeys (*Macaca mulatta*) participated in the experiment. The characteristics of the subjects are listed in Table 1. All subjects were between 7 to 16 years of age (mean = 11.14, SD = 2.6) and had been OVX for an average of 1.5 years. A subset of the monkeys (n=6) had been OVX for about 5 years and had previously participated to cognitive and motor studies with short-term periods of ethinyl estradiol treatment, at least 3 years prior to the present experiment [38,39]. We verified that the performance of these monkeys did not differ significantly from that of the rest of the group in each task and treatment conditions (see Results section). The monkeys were housed in a room adjacent to the experimental room. They were not deprived of food or water and received their normal ration of monkey chow and fresh fruits everyday. The studies were approved by the Institutional Animal Care and Use Committee of Emory University and the monkeys were humanely treated in accordance with the standards of the PHS policy on Humane Care and Use of Laboratory Animals.

Procedure

Cognitive Testing—All monkeys were proficient with the touchscreen and had been trained on the battery of cognitive tasks for several months prior to the experiment, using computer software designed in our laboratory. During the preliminary testing, the monkeys were initially trained to touch a clipart image on the screen in order to receive a reward. Next the battery of cognitive tasks was begun. The monkeys were first trained on the Delayed-Non-Matching-To-Sample task (DNMS). However, only 8 monkeys out of the 21 reached the learning criterion in the task. The 8 monkeys were assigned to different treatment groups and tested on the DNMS with mixed delays (1, 10, 30s) along with the other tasks of the battery, but their performance on the task was not above chance levels. The results concerning the DNMS are therefore not reported in this paper. The final cognitive battery consisted of the spatial-, object-, and face-conditions of the Delayed Recognition Span Tests (DRST) and the Cued Search task.

The 21 monkeys were tested on the cognitive battery 5 days a week for a total of 6 consecutive weeks (Fig. 1). Following a 4 week washout period, a subset of 6 monkeys was re-tested for an additional 4 weeks. Importantly, the 6 monkeys were randomly taken from the EB and DPN groups only, to avoid potential interference with long-term circulation of TAM. In a two-week cycle (hereafter referred to as “block of testing”), monkeys were administered, in this order: the object-DRST (3 consecutive days), the face-DRST (2 consecutive days), the spatial-DRST (2 consecutive days) and the Cued Search task (3 consecutive days). A schematic of each task can be seen in Fig. 2.

Delayed Recognition Span Tests: *Spatial-DRST*: The spatial-DRST is a task of spatial working memory that tests the ability to recognize a novel location among an increasing array of locations. It requires the integrity of the medial temporal lobe [40] and prefrontal cortical systems. We have previously reported that performance on this task is sensitive to ovarian hormones and varies as a function of the menstrual cycle in young females [41], and as a function of long-term ovariectomy [42] and estradiol treatment in aged monkeys [43].

The spatial-DRST required subjects to select the image appearing in a new location among an increasing array of identical images presented one at a time. For this task, the screen was programmed to display 15 non-overlapping positions, arranged in a 3 × 5 matrix. The stimuli were identical clip-art images (randomly drawn at each testing session from a database of 5000 clip-art images), presented on a black background. On the first trial of a sequence, one image appeared in one of the 15 possible positions. The monkey had to touch the image to obtain a

pellet. The screen was cleared and 2 s later, two images were presented: one image reappeared in its original location and an identical image appeared in a new location. The monkey was required to touch the image in the new location to obtain the reward. Each successive correct response was followed by the addition of an identical image appearing in a new location until the monkey made an error, or up to a maximum of 9 images. An error—a touch on an image in a previously seen location—was followed by a green display signaling the onset of a 5s timeout. Ten trials were presented in one session. The exact position of each image at each trial was determined by a random sequence that was unique within and across sessions. The dependent variables were the mean number of locations correctly identified before making an error (spatial memory span), reflecting working memory, and the averaged response times on a stimulus for each trial. Responses times were analyzed to check potential drug-induced sedative effects or increases in activity levels. Chance performance on this task is 1.72.

Object-DRST: The object-DRST is a task of working memory that is sensitive to hippocampal lesions in monkeys [40]. Although estradiol treatment did not affect object-DRST performance in a previous study in young OVX females [38], we incorporated the task into the battery as a control for the two other conditions (spatial and faces). The object-DRST followed the same principles as the spatial-DRST but the stimuli were different images instead of identical images (randomly drawn from a pool of 5000 color clipart objects). The location of each image changed in a random fashion at each trial so that the monkey had to identify the new image based only on visual, rather than spatial cues. We recorded the mean number of objects that the monkey was able to correctly identify before making an error (touching a previously seen object) and the averaged response times.

Face-DRST: Similar rules applied to the face-DRST, but the stimuli were pictures of rhesus monkey faces instead of objects. Performance on Face-DRST decreased during periods of estrogen treatment in a previous study in young OVX monkeys [38]. Since the impairment was only observed for conspecific faces (as opposed to human or chimpanzee faces), the results suggested an effect of estradiol at the emotional or attentional level, specific of the perception/recognition of socially-relevant stimuli. The stimuli were black and white portraits of unfamiliar adult rhesus monkey of both sexes [38]. The pictures were randomly drawn at each session from a pool of 120 pictures. The location of each face changed in a random fashion at each trial so that the monkey had to select the new face based only on visual, rather than spatial cues. We recorded the mean number of faces that the monkey was able to correctly identify before making an error (touching a previously seen face) and the averaged response times.

Cued Search Task (attention): The cued search task was an attentional task that required monkeys to identify a red star randomly appearing among an array of 11 red disks (distracters) at each trial. Two seconds before the appearance of the red star, a bright yellow disk (the cue), the same size as the red star, was briefly flashed for 1 s before clearing. The cue was either congruent with the target, if it appeared at the same location as the red star, or incongruent, if appearing at a different location. Touching one of the distracters was followed by an auditory cue and a 5 s green timeout. Twenty trials were given in one testing session. The cues appeared on every trial in an intermixed fashion for a total of 10 congruent trials and 10 incongruent trials. We recorded the accuracy and the response times. We predicted that if the monkeys were attending to the cue, they should be slower and/or less accurate for incongruent compared to congruent trials. The effect of estrogen treatment were not previously assessed in this particular task, but a study in young OVX monkeys has suggested that estradiol benefits attentional processes [44].

Treatments, blood samples and assays—The first two weeks of the study served as a baseline period after which monkeys were assigned to one of 3 treatment groups: Estradiol

benzoate (EB; Sigma chemical, St Louis, MO; n=7); TAM (Tocris, Ellisville, MO; n=7); the ER- β selective agonist DPN (Tocris, Ellisville, MO; n=7). In each treatment group, half of the monkeys were treated with placebo (sesame oil for 2 weeks) followed by the drug (2 weeks). The other half received the reverse sequence, in a cross-over design. After a wash-out period of 4 weeks, a subset of 6 animals from the EB and DPN groups were re-tested for 4 additional weeks, consisting of 2 weeks of treatment with an ER- α selective agonist PPT (Tocris, Ellisville, MO) and 2 weeks of treatment with oil vehicle (Fig. 1).

Monkeys in each treatment group received daily subcutaneous injections of oil vehicle, EB (2 μ g/kg), TAM (0.48 mg/kg), DPN (0.15 mg/kg) or PPT (0.15 mg/kg). The EB dose was designed to elicit low levels of estradiol, in the early follicular range. The selected TAM dose was based on the high dose typically used in women with breast cancer [45] and on a previous study in female macaques [32]. The doses for DPN and PPT were based on doses used previously in rats [25,26]. Treatment syringes were coded and the treatment codes were broken only at the time of data analysis. The monkeys were trained to extend their leg for conscious venipuncture and blood samples (~3ml) were collected from a saphenous vein twice a week (Monday and Thursday at 8:30 AM). Blood samples were allowed to clot in serum separation tubes and then spun for ten minutes. Serum was frozen for later analyses.

Estradiol assays were performed by the Yerkes Biomarker Core with a commercial radioimmunoassay kit from Diagnostic Systems Laboratories (Los Angeles, CA). TAM assays were performed by the laboratory of Dr. Robert Bonsall (Emory University School of Medicine, Atlanta, GA), by means of HPLC with Fluorescence-UV detection. Assays are not currently available for DPN or PPT.

Analysis

In each treatment group, monkeys were administered either placebo for 2 weeks followed by drug treatment for 2 weeks, or the reverse sequence. Thus, we first examined the effect of treatment sequence on performance for each task (proportion of correct trials or memory spans and response times) using analyses of variance (ANOVAs) with treatment sequence (1, 2) and treatment group (EB, TAM, DPN) as independent variables. Similar analyses were performed separately for the PPT group. Because the effect of treatment sequence and the interaction between group and treatment sequence were not significant for any task or group, treatment sequence was excluded from subsequent analyses.

Performance at baseline (first 2 weeks) was examined for each task using repeated measures analyses of variance (ANOVAs), with treatment Group (EB, TAM, DPN) as a between subject factor. Because the PPT study was performed after the main experiment, it was analyzed separately. Treatment analysis (drug vs. placebo) was performed on the DRSTs and the Cued Search tasks in each treatment group (EB, DPN, TAM and PPT). For the three versions of the DRST (Spatial, Object and Faces), memory spans and response times were examined as a function of Treatment. For the Cued Search Task, the proportion of correct trials and the response times were evaluated in repeated measures ANOVAs using Treatment and Type of Trial (congruent or incongruent) as repeated factors. Because length of estrogen depletion has been suggested to be a major factor in mediating the effects of exogenous estrogens on cognition [46] [47] [48], we verified that the performance of the 6 monkeys with longer periods of OVX did not significantly differ from the performance of the other monkeys for each task and treatment conditions. We included Duration of Ovariectomy (short, long) as a between-subject factor in all the analyses.

RESULTS

Hormonal Assays

Table 2 shows the averaged serum values of E₂ and TAM obtained during drug and oil treatments for the EB and TAM groups.

E₂ levels during periods of EB treatment were significantly higher ($24.21 \text{ pg/ml} \pm 2.93$) than during periods of oil treatment (undetectable levels $< 5 \text{ pg/ml}$; paired t-test, $t(6)=8.26$, $p<.001$). The values were in the range of early follicular levels, when E₂ levels are low. TAM levels were significantly higher during TAM treatment (5.42 ± 0.97) than during oil treatment (2.09 ± 1.20 ; $t(6)=-4.58$, $p<.01$). We verified that TAM levels were significantly lower during oil treatment for monkeys receiving TAM as their first treatment, since the half-life of TAM has been reported to be as long as 7 days [49]. For the 4 monkeys receiving TAM first, levels of TAM were significantly higher during TAM treatment (6.27 ± 1.89 ; $t(3)=-3.44$, $p<.05$) than during oil treatment (3.65 ± 1.19), but TAM was still at detectable levels during the phase of oil treatment suggesting a carry over effect.

Cognitive performance

Spatial-DRST—Baseline performance was $1.79 (\pm 0.036)$ with mean response times of $3.13 \text{ s} (\pm 0.22)$. The span performance was only marginally different from chance ($t_{20}=1.97$, $p<.06$). The groups did not differ at baseline for the memory spans but the DPN group was significantly slower than the other two groups before treatment ($F(2,18)=3.6$, $p<.05$).

As can be seen in Table 3, there was no effect of treatment on the memory span or the responses times in all but the DPN group, for which response times were significantly slower (4.26 ± 0.47) during periods of DPN treatment than during the period of oil treatment (3.41 ± 0.43 ; $F(1,6)=8.41$, $p<.05$). The duration of ovariectomy did not significantly interact with treatment condition for either the memory spans or the response times in any of the treatment groups.

Object DRST—Mean performance at baseline on object-DRST was 1.80 ± 0.024 , and response times were $2.89 (\pm 0.18)$. Spans obtained on the object-DRST were significantly above chance ($t_{20}=3.36$, $p<.01$). The groups did not differ at baseline for the memory spans but the DPN group was significantly slower than the other two groups ($F(2,18)=3.85$, $p<.05$).

Treatments had no significant effect on memory span or response times in either group. The TAM group tended to have greater memory spans with TAM treatment (1.89 ± 0.08) compared to oil (1.76 ± 0.05 ; $F(1,6)=4.32$, $p<.10$). The duration of ovariectomy did not significantly interact with treatment condition for either the memory spans or the response times in any of the treatment groups.

Face-DRST—Mean performance at baseline on Face-DRST was $1.79 (\pm 0.026)$, and response times were $3.47 (\pm 0.38)$. Spans were significantly above chance ($t_{20}=2.73$, $p<.02$). The groups did not differ at baseline for the memory spans but the DPN group was significantly slower than the other two groups ($F(2,18)=3.9$, $p<.05$). Treatments had no significant effect on the memory spans or response times in either group. The interaction between duration of ovariectomy and treatment reached significance for the response times in the EB group. These results indicated that response times were reduced (2.57 ± 1.85) with EB treatment compared to oil (2.89 ± 2.04) in monkeys with short durations of ovariectomy but were increased with EB treatment (2.77 ± 1.63) compared to oil (2.28 ± 0.93) in monkeys with longer durations of ovariectomy ($F(1,5)=13.57$, $p<.05$).

Baseline performance—Monkeys obtained an average of 80% correct responses ($\pm 3\%$) on this task, and responded in an average of 1.95 s (± 0.15). The Type of Trial (congruent, incongruent) did not significantly affect either of these variables at baseline (proportion of correct trials, $F(1,18)=0.52$, ns; response times ($F(1,18)=0.05$, ns). The three groups (EB, TAM, DPN) did not differ significantly at baseline for either the proportion of correct responses ($F(2,18)=0.73$, ns) or the response times ($F(2,18)=1.88$, ns). The subset of monkeys forming the PPT group also had 80% correct responses (± 0.09) and an average of 2.09 response times (± 0.31). They did not show an effect of the Type of Trial for either the correct responses ($F(1,5)=1.59$, ns) or the response times ($F(1,5)=0.04$, ns).

Treatment Effects—The effects of treatments for each of the 4 treatment groups can be seen in Fig. 3 (proportion correct) and Fig. 4 (response times)

EB: The main effects of Treatment ($F(1,6)=1.07$, ns) and Type of Trial ($F(1,6)=0.42$, ns) as well as the interaction ($F(1,6)=0.001$, ns) were not significant for either the proportion of correct responses or the response times (Treatment: $F(1,6)=0.22$, ns; Type of Trial $F(1,6)=0.6$, ns; Treatment $\times$ Type of Trial $F(1,6)=0.003$, ns). The duration of ovariectomy did not significantly interact with treatment for either accuracy or the response times.

TAM: The main effects of Treatment ($F(1,6)=0.35$, ns) and Type of Trial ($F(1,6)=0.19$, ns) were not significant for either the proportion of correct responses or the response times (Treatment: $F(1,6)=0.26$, ns; Type of Trial ($F(1,6)=0.26$, ns). The interaction between Treatment and Type of Trial on the proportion of correct trials was of borderline significance ($F(1,6)=4.98$, $p<.10$). The interaction was not significant for the response times ($F(1,6)=2.67$, ns). The duration of ovariectomy did not significantly interact with treatment for either accuracy or the response times

DPN: The main effects of Treatment ($F(1,6)=0.024$, ns) and Type of Trial ($F(1,6)=0.029$, ns) as well as the interaction ($F(1,6)=1.39$, ns) were not significant for either the proportion of correct responses or the response times (Treatment: $F(1,6)=1.8$, ns; Type of Trial $F(1,6)=0.067$, ns; Treatment $\times$ Type of Trial $F(1,6)=0.54$, ns). The duration of ovariectomy did not significantly interact with treatment for either accuracy or the response times.

PPT: Monkeys in the PPT group tended to obtain slightly lower scores for incongruent (83% correct) than for congruent trials (85% correct; $F(1,5)=5.88$, $p=.06$). The effects of Treatment ($F(1,5)=0.19$, ns) and the interaction between Treatment and Type of Trial ($F(1,5)=0.19$, ns) were not significant, for either the proportion of correct trials or the response times (Treatment: $F(1,5)=0.89$, ns; Type of Trial $F(1,5)=1.35$, ns). The duration of ovariectomy did not significantly interact with treatment for either accuracy or the response times

DISCUSSION

We tested adult OVX female rhesus monkeys on tasks assessing working memory load and attention, during treatment for 2 weeks with EB, DPN (an ER β agonist), TAM (a non selective ER antagonist), PPT (an ER α agonist) or oil vehicle, in a cross-over design. The study is the first to test selective ER agonists and antagonist on cognitive performance in nonhuman primates. Overall, treatments had only negligible or no effect on cognition. These results are consistent with accumulating evidence that estrogens do not significantly affect memory function in young adult OVX monkeys (see for review [5]), and suggest further that the lack of effect is independent of the type of estrogens, mode of administration, duration of treatment or regimen used. Indeed, studies using oral ethinyl estradiol in alternating months [38], implants of 17 β estradiol for several months [50], injections of estradiol cypionate every 3 weeks [51]

or injections of estradiol benzoate and other ER ligands for 2 weeks (this paper), all have failed to alter memory in young OVX monkeys.

The interpretation of negative findings is inherently challenging, as many factors could underlie the inefficacy of the selected treatments on cognition. First, it may be argued that the relative heterogeneity of our sample may have affected the results. Indeed, 6 monkeys in the group had been OVX for a period of about 5 years while the other monkeys had experienced less than a year of estrogen deprivation prior to the start of the experiment. Since it has been suggested that duration of estrogen depletion may mediate the effects of estrogens on cognition [46,47, 52], we took into account the differential duration of ovariectomy in our analyses. Overall, the length of ovariectomy had only very small effects on performance, affecting only responses times in the EB group in the Face-DRST. Thus, it is unlikely that the duration of ovariectomy played a major role in shaping our results. We recognize however that a larger sample would be needed to adequately test this hypothesis.

Second, the duration of treatment (2 weeks) may have been too short to significantly affect cognitive performance. However, data from the human literature argue against such an interpretation, as 3 weeks [53], 2 weeks [54] or even 3 days of treatment with estradiol [55] have been shown to elicit changes in cognition in women. Thus, the short duration of treatment is unlikely to have played a major role in the present results.

Another potential explanation for the lack of estrogen effect on cognitive performance concerns the hormonal doses used. In the present study, we used a low EB dose, yielding serum estradiol levels in the early follicular range. We selected this dose with regards to clinical applications, as low-dose estrogen formulations are now encouraged for safer use of hormonal therapy in women [56]. This dose may have been too low to impact cognitive performance. Yet, since all the cognitive studies in nonhuman primates so far have used treatments producing moderate to high levels of circulating estradiol, the role of the dose in influencing cognitive outcome remains uncertain. Nevertheless, dose differences could explain some of the discrepancies between the present results and those of previous studies in young OVX macaques. Indeed, in contrast to the studies of Voytko (2002) and Lacreuse and Herndon (2003), two studies in which estradiol levels were in the periovulatory range, we failed to observe a (beneficial) effect of estradiol on attention and a (negative) effect of estradiol on Face-DRST, respectively. The attention task that we used was different from that of Voytko (2000), and may not have been as sensitive. The Face-DRST, however, was identical to that of Lacreuse and Herndon (2003) and the failure to replicate the negative impact of estrogens on this task could be due to dose differences. Note that in the present study the same trend as in Lacreuse and Herndon (2003) was observed, i.e., monkeys obtained lower spatial memory spans when treated with EB compared to oil, yet, the difference did not reach significance. Although other factors besides the dose may have played a role (the type of estrogen, route of administration, duration of treatment were all different between the two studies), studies in rodents indicate that low vs. high doses of estradiol can have a large impact on the kind of cognitive responses elicited [57-59]. In addition, the few studies that have used very low doses of estrogens in postmenopausal women have concluded that they do not significantly alter cognitive function [60,61].

Whether or not the EB dose was an issue, the selected doses for DPN and PPT (0.15 mg/kg) were based on doses shown to be effective in modulating cognitive performance and anxiety in rats [25,26]. The dose selected for TAM (0.48 mg/kg/day) corresponded to a clinically relevant dose and was also the dose shown to significantly increase anxiety in female rhesus monkeys [32,33]. Thus, treatment with the selected doses of ER ligands was predicted to impact mnemonic and attentional function. In contrast to our hypothesis, TAM and PPT treatments did not differ from oil in any of the tasks; the effect of DPN was limited to slower response

times in only one task. The significance of this latter result is unclear, in light of the facts that the DPN group was slower than the other groups at baseline and that the effects of the drug were only observed for the spatial version of the DRST. Thus, it is unlikely that DPN induced overall sedative-like effects. Rather, one interpretation of this difference, based on the rodent literature, is that monkeys might have experienced reduced anxiety with DPN compared to oil in this particular task. Indeed, DPN has been shown to decrease anxiety-like and depression-like behaviors in rats and mice in certain tasks [24-26,62]. Yet, alternative explanations, such as decreased motivation or increased difficulty with spatial processing, are plausible and more studies are needed to understand the significance of this effect.

In conclusion, none of the ER ligands used in the study, EB, DPN, PPT or TAM had a significant impact on cognitive function in this cross-over study in adult females rhesus monkeys treated for 2 weeks. These results contrast sharply with recent findings from the rodent literature which clearly show an impact of these agents on learning and memory processes. In OVX rats, EB has been shown to affect working memory [63], DPN to enhance object recognition, object location [18] and spatial memory [22], and PPT to enhance lordosis [22]. While fewer studies have been conducted on TAM in rodents, at least one study in mice found impairment in spatial learning following TAM treatment [64]. The basis for these species differences in the cognitive responses to estradiol and SERMs remains to be understood. One possibility relates to differences in the relative duration of ovariectomy between rodents and monkeys. Monkeys are typically OVX for a longer time than rodents prior to treatment and this disproportionately longer period of estrogen deprivation could interfere with the ability of estrogenic drugs to influence cognition. Yet, this hypothesis does not account for the pattern of species differences that emerges with aging: cognitive function tends to become more sensitive to estrogens in aged monkeys [5,51], while the reversed pattern is observed in aged rodents [65]. Thus, if time since ovariectomy plays a role, it is certainly not the only factor mediating species differences. An alternative hypothesis, speculates that the rodent and monkey brains are differentially affected by changes in cerebral production of estradiol during the course of aging [66]. Additional studies are needed to validate this intriguing possibility.

In conclusion, the results of this study add to the evidence that memory is not significantly affected by estrogen manipulations in young female monkeys [5] and suggest furthermore that SERMs have a minimal impact on cognition at this life stage. Because estrogens affect cognitive abilities in aged monkeys however, similar studies should be carried out in older females to examine whether estrogen effects in the old brain are mediated by ER β or ER α or by complex interactions between the two receptors [67].

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Figure 1.
Design and timeline of the experiment

Figure 2. Schematic of the cognitive tasks. The cross represents the correct stimulus

a. Delayed Recognition Span Tests (DRSTs). Spatial version: the monkey must select the stimulus appearing in a new location; Object version: the monkey must select the new object; Face version: the monkey must select the new rhesus monkey face (only the first 3 trials of a sequence are represented). **b.** Cued search task: Two seconds before the appearance of target (star) a cue flashes for 1s. The cue is congruent if it predicts the location of the star, or incongruent, if it appears at a different location. The monkey must touch the target to be rewarded.

Figure 3.

Proportion of correct responses in the Cued Search Task as a function of treatment with oil or drug in each of the 4 treatment groups.

Figure 4.

Response times in the Cued Search Task as a function of treatment with oil or drug in each of the 4 treatment groups.

Table 1
Characteristics and treatment group of the 21 female rhesus monkeys. OVX: ovariectomy.

Monkey Code	Hormonal Treatment Group	Age at test (years)	Age at OVX (years)	Length OVX (years)
RRr6	DPN	7.75	7.75	0.00
RZm6	DPN	8.00	7.92	0.08
RLc6	DPN	8.83	8.83	0.00
RNm5*	DPN	10.58	5.33	5.25
RRd5	DPN	10.83	10.83	0.00
RLd4*	DPN	12.83	7.58	5.25
RGc4	DPN	13.08	13.00	0.08
RTb6	EB	8.83	8.83	0.00
RRc5*	EB	10.92	5.67	5.25
RPu4*	EB	11.83	6.50	5.33
RMo4	EB	12.58	12.42	0.17
RWv3	EB	13.92	13.83	0.08
Rce3	EB	15.08	14.92	0.17
REv2	EB	16.00	15.92	0.08
RDe7	TAM	6.75	6.75	0.00
RVo6	TAM	7.75	7.75	0.00
RAk6	TAM	8.08	7.92	0.17
REj4	TAM	10.75	10.75	0.00
RJg4*	TAM	12.83	7.50	5.33
RDf4	TAM	13.00	12.92	0.08
RFr3*	TAM	13.83	8.58	5.25
Mean		11.14	9.59	1.55
SD		2.60	3.04	2.36

Averaged levels of estradiol and tamoxifen ($\pm$ SEM) in monkeys treated with EB or TAM compared to oil vehicle, paired student t test, and p value.

Table 2

Group	Assays	Drug	Oil	t	p
EB (n=7)	Estradiol (pg/ml)	24.21 (2.93)	<5	8.26, df =6	p<.0001
TAM (n=7)	Tamoxifen (ng/ml)	5.42 (0.97)	2.09 (1.20)	4.58, df =6	p<.01

Table 3

Mean memory spans and response times (SEM), degrees of freedom (df), F statistic and p-value in the Spatial-DRST (top), Object-DRST (middle) and Face-DRST (bottom) when monkeys were treated with oil or the drug in each of the 4 treatment groups (EB: estradiol benzoate; TAM: tamoxifen; DPN: diarylpropionitrile; PPT: propyl-pyrazole-triol); Chance level for the memory span is 1.72.

Spatial-DRST		Oil	Drug	df	F	P
Memory Span	EB	1.82 (0.07)	1.78 (0.08)	6	0.19	Ns
	TAM	1.77 (0.04)	1.80 (0.09)	6	0.09	Ns
	DPN	1.88 (0.07)	1.81 (0.04)	6	1.18	Ns
	PPT	1.75 (0.08)	1.87 (0.07)	5	1.74	Ns
Response Times (s)	EB	2.62 (0.14)	2.42 (0.17)	6	0.75	Ns
	TAM	2.53 (0.35)	2.87 (0.55)	6	1.45	Ns
	DPN	3.41 (0.43)	4.26 (0.47)	6	8.41	p < .05
	PPT	3.07 (0.38)	3.66 (0.9)	5	0.44	Ns
Object-DRST		Oil	Drug	df	F	P
Memory Span	EB	1.78 (0.04)	1.80 (0.07)	6	0.041	Ns
	TAM	1.76 (0.05)	1.89 (0.08)	6	4.32	0.08
	DPN	1.78 (0.06)	1.79 (0.06)	6	0.020	Ns
	PPT	1.79 (0.10)	1.93 (0.06)	5	2.49	Ns
Response Times (s)	EB	2.75 (0.23)	2.38 (0.11)	6	2.31	Ns
	TAM	2.77 (0.36)	2.67 (0.34)	6	0.049	Ns
	DPN	3.51 (0.40)	3.53 (0.40)	6	0.004	Ns
	PPT	2.94 (0.36)	3.23 (0.35)	5	1.42	Ns
Face-DRST		Oil	Drug	df	F	P
Memory Span	EB	1.79 (0.07)	1.67 (0.07)	6	1.14	Ns
	TAM	1.69 (0.05)	1.80 (0.06)	6	3.22	Ns
	DPN	1.72 (0.06)	1.73 (0.03)	6	0.06	Ns
	PPT	1.69 (0.04)	1.62 (0.04)	5	2.29	Ns

Spatial-DRST		Oil	Drug	df	F	P
Response Times (s)	EB	2.70 (0.35)	2.63 (0.22)	6	0.07	Ns
	TAM	3.07 (0.67)	2.67 (0.46)	6	1.57	Ns
	DPN	3.66 (0.61)	3.92 (0.50)	6	0.30	Ns
	PPT	3.45 (0.61)	3.50 (0.75)	5	0.08	Ns