Published in final edited form as:

J Neuroendocrinol. 2014 May ; 26(5): 296–309. doi:10.1111/jne.12147.

Oestradiol modulation of cognition in adult female marmosets (*Callithrix jacchus*)

Agnès Lacreuse^{1,*}, Jeemin Chang¹, Christina M. Metevier¹, Matthew LaClair², Jerrold S. Meyer¹, and Craig M. Ferris³

¹Department of Psychology, University of Massachusetts, Amherst MA 01003

²Neuroscience and Behavior graduate program, University of Massachusetts, Amherst MA

³Department of Psychology, Northeastern University, Boston MA 02115

Abstract

The common marmoset (*Callithrix jacchus*) provides many advantages over traditional rodent and macaque species as a model for human aging and may be very valuable to study the effects of sex steroids on cognitive and brain aging. We present the first study examining the effects of oestrogens on cognitive function in female marmosets. Adult monkeys (3-5 years of age) were trained to a specific learning criterion on a battery of cognitive tasks preoperatively (object discrimination, delayed response with increasing delays and detour reaching with opaque box) and tested on different versions of these tasks (object reversals, delayed response with randomised delays and detour reaching with clear box) following ovariectomy and simultaneous implantation with 17 β -oestradiol (E₂, n=6) or blank (n=6) Silastic capsules. Acquisition of a delayed matching-to-position task with a 1s delay was also administered following completion of these tests. E₂-treated monkeys were significantly impaired on the second Reversal and showed an increase in perseverative responding from Reversals 1 to 3. Their performance also tended to be worse than that of control monkeys on the Delayed Response task. Performance acquisition on the DMP tended to be better in E₂-treated relative to control monkeys, but the group difference did not reach statistical significance. No effect of treatment was detected for Detour Reaching or affiliative behaviours. Overall, the findings indicate that E₂ compromises performance on prefrontally-mediated tasks. The suggestion that E₂ may improve acquisition on tasks dependent on the hippocampus will require further validation. These results are discussed in the context of dopaminergic and serotonergic signaling. We conclude that the marmoset is a useful new primate model to examine the effects of oestrogens on cognitive function.

Keywords

Aging; menopause; learning and memory; ovariectomy; oestrogens

*Correspondence to: Department of Psychology 135 Hicks Way University of Massachusetts Amherst, MA 01003 Phone: 413-545-2183 Fax: 413-545-0996 alacreuse@psych.umass.edu.

INTRODUCTION

Cognitive deficits associated with normal aging are large and widespread in humans. While a few cognitive domains are spared or even increased by age, such as vocabulary and some general abilities learned early in life (1), most cognitive domains follow a linear decline with age, including working memory, speed of processing, executive function, declarative memory and reasoning (1, 2). Age-related cognitive impairments are associated with a decreased ability to function in daily life (3). In the context of a rapidly aging population Worldwide (4), it is crucial to develop treatments that alleviate age-related cognitive dysfunction and associated functional limitations.

Sex hormones also decline with age in both men (5) and women (6) and it has long been thought that changes in the hormonal milieu may contribute to cognitive impairment (7-9). As a result, hormonal replacement therapy (HRT) was investigated as a way to alleviate age-related cognitive decline, particularly in postmenopausal women. Many small randomised controlled trials and observational studies in women (10) as well as data from animal studies (11) provided evidence for a beneficial effect of oestrogens on at least some cognitive function. However, enthusiasm for HRT was halted following the publication of the main findings of the Women's Health Initiative (WHI) study and those of two ancillary studies, which showed that chronic administration of a combination of conjugated equine oestrogens (CEE) and Medroxy Progesterone Acetate (MPA) was harmful to older postmenopausal women for a number of health outcomes (12), as well as cognitive function (13) and dementia risk (14). Ten years later, after much work and controversial debate over the unexpected results of these trials, the potential of HRT as an intervention against age-related cognitive decline remains to be fully understood (15).

Translational animal models are necessary for understanding the mechanisms of oestrogen action on the aging brain, predicting the therapeutic effects in women and designing new therapeutics for women's cognitive health (16, 17). Rodents (mice and rats) and a few species of nonhuman primates (macaques) are currently the animal models of choice for studying the neurocognitive effects of ovarian hormones. Rodents have provided a wealth of information about the effects of oestrogens on the brain and cognition but are phylogenetically distant from humans and present significant differences in endocrinology, brain organisation and cognitive function that limit their translational impact. These limitations are minimised in macaque species (*Macaca mulatta* or *M. fascicularis*) which share many characteristics with humans in terms of endocrine physiology, cognition, neuroanatomy, and social complexity. Macaques have proven to be essential for translational research in neuroscience (18), including in studies relevant to women's cognitive health (19-21). Yet, research in macaques is weakened by a number of shortcomings (22). First, macaques have a long lifespan (maximum of 40 years) that is not well suited for longitudinal investigations of the effects of oestrogens on brain function. Second, the limited availability of aged macaques makes aging research on these species difficult. Third, their high cost limits the number of animals that can be studied, leading to studies with small sample sizes. In addition, special precautions must be followed with animals that carry serious zoonotic disease.

We have begun to study the neurocognitive effects of HRT in a small New World primate recently identified as a useful model for human aging (23-25), the common marmoset (*Callithrix jacchus*). The marmoset is the shortest-lived anthropoid primate, with an average lifespan in captivity of 4-6 years and a maximum lifespan of 16 years (26, 27). These estimates may depend on a variety of contextual factors, as longer estimates of 10 years and 21.7 years, respectively, have recently been reported in a Japanese colony (28). Nevertheless, marmosets 8 years of age are considered aged, due to the appearance of aging signs such as cartilaginous changes in intra-articular discs, hearing loss, loss of calbindin D28k binding from basal cholinergic neurons (reviewed in (27)), reduced neurogenesis in the hippocampus (29) and β -amyloid deposition in the cerebral cortex (30). The very small size of the marmoset (300-500g), its high reproductive rate, minimal biosafety concerns, relative low cost and ease of maintenance in the laboratory have made it an attractive model for biomedical research (27). Its use as a model for human health and disease will likely increase in the coming years, following completion of the sequencing of its genome and the further development of transgenic marmosets (31).

The marmoset has a large brain that represents 2.7% of its body weight (32) and that is about 5 times larger than the brain of a rat of similar size (27). However, it is about 10 times smaller than the macaque brain and 180 times smaller than the human brain. Despite these large differences in brain volume, a similar layout of homologous sensory, motor and associations areas can be observed among the 3 species. Yet, some areas are disproportionally larger in the human brain. For example, the prefrontal cortex (PFC) occupies a larger proportion of brain volume, shows increased branching complexity in specific layers, and has greater white matter volume in humans than in other primates (reviewed in (33)). A recent analysis of cerebral cortex expansion between marmoset, capuchin and macaque brains using computational methods has identified the temporal parietal junction, ventrolateral PFC and the dorsal anterior cingulate cortex as the most prominent centers of expansion from marmosets to macaques (34). These expansion sites are similar to those identified between the macaque and the human brain. The most striking difference between the marmoset and the human brain is the massive expansion of the temporal parietal junction area, an area involved in speech, morality and theory of mind. The ventrolateral PFC is typically associated with working memory and behavioral flexibility, while the dorsal anterior cingulate cortex is primarily involved in emotion processing, reward processing and problem solving. Thus, these areas of expansion may support major differences in higher cognitive processing between marmosets and humans. A variety of neuroimaging techniques have been successfully used in marmosets and several brain atlases are now available (35, 36). Importantly, conscious marmosets can be successfully imaged with functional magnetic resonance imaging (fMRI), using noninvasive procedures that require minimal training (37, 38). Using resting-state fMRI in awake marmosets, Belcher and collaborators have recently identified 12 networks that overlap substantially with known circuits observed in the awake human (39). These data suggest that the marmoset is an appropriate model for human brain function. Marmosets are able to perform a wide variety of cognitive tasks, using manual (e.g., (40)) as well as automated touchscreen systems (41, 42). The marmoset is phylogenetically more distant from humans than macaques (the split between New World monkeys and Old World monkeys is estimated to have occurred around

35 million years ago), and their cognitive abilities do present certain limitations compared to macaques. For example, acquisition of delayed non matching-to-sample tasks has been reported to be particularly difficult in this species (41). Nevertheless, it has been argued that the cognitive performance of marmosets, as assessed by the transfer index on reversal learning is better than would be expected given their brain size and surpasses that of larger New World monkeys such as capuchins (43).

The endocrine physiology of the female marmoset has been extensively studied. Like rhesus monkeys and humans, marmosets have an ovarian cycle of about 28 days, divided into a follicular period (follicular development), an ovulation phase (surge in oestradiol and chorionic gonadotropin hormone) and a luteal phase (rise in progesterone levels; (44)). However, the plasma concentrations of oestrone and progesterone are 6-20 times higher than in Old World monkeys (44) and humans. Marmosets display ovarian aging, characterized by follicular depletion and anovulation, but they do not experience menopause, due to the presence of persistent steroidogenic interstitial glands in the ovary (45). This does not diminish the value of the marmoset as a model for menopause, which can be modeled through ovariectomy. It is important to note that although female rhesus macaques experience menopause, it occurs very late in their lifetime (> 24 years of age; (46). As a result, all the studies focusing on the cognitive effects of HRT in nonhuman primates have been conducted in ovariectomised (OVX) monkeys, as opposed to naturally menopausal monkeys.

In summary, the physiological, cognitive and lifespan characteristics of the marmoset make it a particularly promising model for examining the neurocognitive effects of hormonal therapy. The study presented here is the first to examine the validity of the marmoset as such a model.

MATERIALS AND METHODS

Subjects

Twelve adult female common marmosets (*Callithrix jacchus*), 2.5-5 years of age (mean = 3, SD = 0.92) and weighing between 350–550 g participated in the study. The monkeys were experimentally naïve at the start of the experiment. The marmosets were housed indoors, with lights on from 0700 to 1900 h, ambient temperature at 27 °C, and humidity at approximately 50%. Each female was socially housed with an adult male to promote psychological well-being. Males had been castrated to prevent breeding and for the needs of another study at least 2 months prior to housing with the females. The pairs were formed approximately one month prior to the beginning of the study. The pairs were housed in stainless steel mesh cages measuring 40×30×31 inches or 101×76.2×78.74 cm. The cages were furnished with perches, platforms, nest boxes, and hammocks to promote species typical behaviour including foraging, scent-marking, and climbing.

Marmosets were fed of Mazuri Callitrichid High Fiber Diet 5M16 (Purina Mills, St Louis, MO) supplemented with a variety of fresh fruit, nuts, and mealworms. Fruit and nuts were provided twice daily (0800h-0900h and 13h00-1500h). Mazuri was provided between 1300h and 1500h, following cognitive testing, and water was available *ad libitum*. The monkeys

were provided with daily enrichment, including foraging tubes and a variety of toys, in accordance with the federally mandated Environmental Enrichment Program. The animals were cared for in accordance with the guidelines published in the Guide for the Care and Use of Laboratory Animals, 8th edition. The studies were approved by the UMass Institutional Animal Care and Use Committee.

Procedures

Behavioural Assessments—During the behavioural observations, individuals to whom the marmosets had been previously acclimated observed all females in their home cages. All observers were trained by the lab technician (J. Chang) and passed a test of 90% inter-rater reliability over three test sessions before taking behavioral data for the study. The observers recorded the behaviour of focal animals using a modified frequency scoring system. Each monkey was observed twice a day, five days a week, in the morning between 10h00 and 11h00 and afternoon between 16h00 and 17h00 throughout the study. An observer recorded the occurrence of 25 different behaviours in 15 s intervals for 5 min. Behaviours included measures of activity, feeding, social interactions, and exploration based on an extensive ethogram developed specifically for the common marmoset (47); see Table 1).

Cognitive tasks

General procedure: For cognitive testing, monkeys were trained to enter a transport box ($34.1 \times 20.65 \times 30.8\text{cm}$) affixed to their home cage. The front of the box was made out of a mesh through which the monkey could pass her hands and arms. The monkey sat in the transport box in front of a custom-made miniature version of the Wisconsin General Testing Apparatus (WGTA). The modified WGTA was an opaque box ($43.2 \times 42.3 \times 44.5\text{ cm}$) containing a test tray ($40.65 \times 11.15 \times 1.25\text{ cm}$) with two or four food wells (each 2.5 cm in diameter). The wells could be baited with desirable food, such as small pieces of marshmallows, and could be covered by stimulus objects. Between trials, the tray was concealed from view by an opaque screen.

Selection of the cognitive tasks was based on feasibility aspects, putative sensitivity to ovarian hormones and involvement of specific brain regions. The battery of cognitive tasks consisted of the Object Reversals, the Delayed Response, the Detour Reaching task and the acquisition of the Delayed Matching-to-Position (DMP), administered in this order. This specific task order was selected in order to facilitate learning. Monkeys learned the first 3 cognitive tasks preoperatively. Specifically, they reached a learning criterion on a simple object discrimination task, on each delay of the Delayed Response task, and on the opaque box version of the Detour Reaching task when normally cycling. Once these tasks were learned, they were OVX and implanted with empty implants or implants containing 17β -oestradiol (E_2) (see below). Four weeks following ovariectomy, the test versions of the tasks were administered. Before re-testing, we verified that each monkey was still at criterion level on the previous tasks. The monkeys learned the DMP after completion of these 3 tasks, that is, post-ovariectomy and treatment (Fig. 1). All experimenters were blind to treatment assignments.

Object Reversals: This task has been reported to involve the orbitofrontal cortex (48), the medial striatum (49) as well as ventrolateral PFC (50). It assesses the monkey's capacity to inhibit a response pattern with changing reward contingencies. Our procedure was based on the methods described by Ridley and collaborators (51). To start the test, monkeys were presented with a pair of 3D objects, a white sphere and a black star, over the lateral wells of the testing tray. The two objects were randomly placed over the left or right wells. *Training (preoperatively):* for the initial object discrimination, the black star was always rewarded. Monkeys had to select the black star to find the reward until they reached a criterion of 90% correct responses over 2 consecutive sessions (maximum of 2 errors in 20 trials). Immediately after reaching this learning criterion, they moved on to learn the Delayed Response and Detour Reaching tasks. The test condition (object reversals) occurred post-surgery. *Testing (post-ovariectomy and treatment):* 4 weeks following ovariectomy and treatment, the first reversal was administered: the black star was no longer rewarded and the sphere was now rewarded. Animals were retested for 10 trials per day until they reached a learning criterion of 90% correct responses over 2 consecutive sessions. Three such reversals were administered. The total number of trials and number of errors to reach criterion were analysed. In addition, we examined the types of errors made during each reversal. Following the procedures detailed in Lai and collaborators (52), for each 10 trial session, we calculated the number of perseverative errors (when the number of errors is significantly above chance, 7 to 10), the number of errors when the animal performs at chance levels ("chance errors", 4-6) and the number of errors during the learning stage ("learning errors", when the number of errors is significantly below chance, 0-3).

Delayed Response: The Delayed Response involves the dorsolateral prefrontal cortex (53). Performance on the Delayed Response has been shown to be improved by E₂ treatment in aged OVX rhesus monkeys in one study (54) but not others (55, 56). In the Delayed Response task, monkeys observed the experimenter bait one of two lateral wells with a food reward and cover the wells with identical stimuli (opaque tokens). The tray was concealed from view for a specific delay and then represented to the monkey. The monkey had to select the token covering the reward. We used a procedure based on that used by Collins and collaborators (57) in marmosets with delays of 0, 1, 3, 6, and 10s. Monkeys were tested 10 trials per day, 5 days a week. *Training (preoperatively):* monkeys were trained to a 90% correct responses learning criterion on each successive delay. *Testing (post-ovariectomy and treatment):* monkeys were tested with all delays mixed in a single session (2 trials per delay) for a total of 100 trials (10 days of testing). The percentage of correct responses was analyzed.

Detour-Reaching: The Detour-Reaching task has been used to assess prefrontal dysfunction in monkeys, including marmosets (58). The procedure was based on the method established in Wallis et al. (58). *Training (preoperatively):* the marmoset was presented with an opaque detour box (7.6 × 7.1 × 7.5 cm). One side of the box contained an opening (6.15 × 6.4 cm) through which the monkey could reach with one hand to retrieve a marshmallow. Initially, the open side of the box faced towards the subject, but it was gradually turned in successive sessions from 45° to 90° away from the subject. Training continued until the animal successfully retrieved the reward from the 90° position in either direction. Animals were

tested for 6 trials per day, 5 days a week. *Testing (post-ovariectomy and treatment)*: for testing, a transparent box was used, making the reward visible. The monkeys had to inhibit prepotent reaches directly toward the reward. A total of 10 sessions of 12 trials were given. The dependent variables were the number of reaching errors before getting the reward and the latencies to get the reward.

Acquisition of the Delayed Match-to-position (DMP): The DMP tests object location working memory by requiring the monkey to discriminate a familiar from a novel location following a specific delay. The DMP involves the interaction of the hippocampus (59, 60) and prefrontal cortex with unclear findings regarding the precise contribution of each region to performance (see for review, (61)). Some of the discrepancies are inherent to the attributes of the task administered, as several versions of the DMP exist in the literature. Most commonly, rodents are presented with one of two levers as the sample and have to press the lever that matches the sample after a specific delay. Our version of the DMP involved remembering the sample location relative to that of a foil among 4 possible locations and therefore involved pattern separation. Two recent rat studies using a non-matching version of the DMP with multiple locations presented on a touchscreen (62, 63) have clarified the role of the hippocampus and prefrontal cortex to performance in this type of task. They showed that the hippocampus and medial prefrontal cortex are essential for the retention of memories across a 6s delay, while only the hippocampus is required for spatial pattern separation. According to these results, our paradigm with 4 locations, for which pattern separation is essential, is likely to involve the hippocampus. However, since task acquisition was not examined in the lesion studies mentioned above, it is also possible that both the hippocampus and prefrontal cortex contribute to learning the rule of the task (e.g., (64). E₂ replacement in OVX female rats has been shown to enhance the acquisition of the DMP in a T-maze, via a mechanism that may involve the hippocampus and/or the prefrontal cortex (65).

The acquisition of the DMP was performed following completion of the three tasks described above. *Training (post-ovariectomy and treatment)*: Monkeys were presented with one opaque token over one of 4 wells. The tray was concealed from view for 1s, after which the tray was represented to reveal the sample token over the same well and an identical token over a different well. The animal had to displace the token in the original location to retrieve the food reward. All 4 positions were used and the position of the token at each trial was pseudorandomised. Subjects were tested for 6 trials per day, 5 days per week until a learning criterion of 90% correct responses over 2 consecutive sessions (12 trials), or a maximum of 350 trials. We examined the number of trials and the number of errors to criterion, as well as percentage accuracy as a function of spatial pattern separation. For the analysis of spatial pattern separation, 3 conditions were examined. The Close condition characterized trials in which the two tokens were placed in adjacent positions. In the Medium condition, the two tokens were separated by one well, and in the Far condition, the tokens were separated by 2 wells. Because of the possible stimuli combinations on the 4-well tray, there were more Close trials than Medium and Far trials. Each block of 12 trials was composed of 6 Close trials, 4 Medium trials, and 2 Far trials.

Ovariectomy and implantation of Silastic capsules—Once monkeys reached criterion on each of the first 3 cognitive tasks (except DMP), they were OVX and implanted with subcutaneous Silastic capsules that were either empty (control group, $n = 6$) or contained crystalline 17β -E₂ (Sigma-Aldrich, St Louis, MO; E₂ group, $n = 6$). Prior to treatment group assignment, we verified that there was no significant difference between the controls and E₂-treated monkeys in age (3.56 vs. 3.68 respectively, $t(10) = -0.20$, ns) or learning abilities (number of trials to acquire the initial object discrimination, 44.5 vs. 47.5, $t(10) = -0.24$, ns; to acquire the Delayed Response (averaged across delays): 53.27 vs. 56.5, $t(10) = -0.17$, ns).

For bilateral ovariectomy, females were anaesthetized with ketamine (15mg/kg, i.m.) and maintained on isoflurane (2%; 0.6 L/min oxygen). At the time of ketamine administration, 0.02-0.04 mg/kg atropine i.m. and 0.01mg/kg of buprenorphine i.m. were administered. Each ovary was isolated through a ventral midline incision and exteriorized for visualization of the fallopian tube and ovarian pedicle. The capsules were implanted at the time of ovariectomy through a small dorsal midline skin incision between the scapulae. The incision was closed with surgical tissue glue or sutures. Six monkeys were implanted with two 17β -E₂ Silastic capsules (Dow Corning; I.D: 0.058 in.; O.D: 0.077 in., length 11 mm) and 6 monkeys were implanted with 2 empty capsules. The E₂ implants were expected to yield mid-follicular E₂ levels for at least 10 weeks (66). Monkeys were allowed to recover from ovariectomy for 4 weeks, after which they were tested on each cognitive task and observed for behavioural changes associated with drug treatment. Testing post-surgery took place over a total of 23 weeks. The capsules were replaced at 11 weeks post-ovariectomy with ketamine and isoflurane anaesthesia, as described above. The monkeys were not tested on that day. Following completion of the study, the capsules were removed from all monkeys.

Urine collection, blood collection and oestrogen assays—Urine was collected once a week from all the females throughout the experiment, using the methods described in Saltzman et al. (67). For urinary collection, marmosets were captured from their nest boxes at 06:30h, a few minutes before the lights came on, and immediately placed in a urine-collection chamber until they urinated, or until 1 hour elapsed. Urine was spun for 5 min and placed in 1.5 ml vials and frozen at -20°C . Because of unforeseen problems with the urine assays, we also collected two blood samples at weeks 18 and 19 of the experiment for oestrogens determination from plasma. For blood collection, monkeys were lightly anesthetized with isoflurane and blood was drawn (approximately 0.4 ml) from a saphenous vein. The blood was spun for 15 min and the plasma placed in 1.5 ml vials and frozen at -20°C until assay by the Assay Service Unit of the Wisconsin National Primate Research Center, University of Wisconsin Madison, Madison WI. E₂ was measured according to the methods reported in Saltzman et al., 1998 (68) but without celite chromatography to separate oestrone. Up to 150 μl of plasma were aliquoted for the assay. Samples were extracted with 5 ml of ethyl ether and run by radioimmunoassay.

Statistical analysis—Based on the hormonal data, one control subject with abnormally high levels of oestrogens was eliminated from the analysis. All the analyses are therefore reported for a total of 11 subjects ($n = 6$ in the E₂ group and $n = 5$ in the control group).

Behaviour: The analysis of behaviour was performed for days in which data were available for all 11 monkeys. The dataset consisted of afternoon records totaling 42 days per animals and sampling each month of the experiment. We examined (1) the average daily occurrence of all affiliative behaviours, which included the amount of genital contacts, social contacts, nuzzle sniffing, mounts, social play and social groom initiated and received by the female; (2) the average daily occurrence of affiliative behaviours initiated by the female (genital, social, sniffs, mounts and social groom) and (3) the average daily occurrence of affiliative behaviours received by the female. These measures were compared using independent student t-tests. In addition, we also compared behaviours occurring within the first 7 days vs. the last 7 days using a mixed repeated measures analyses of variance (ANOVA) with Time (first, last) and Group (control, E₂) as factors in order to examine whether the duration of E₂ treatment or ovariectomy influenced the patterns of affiliative behaviours.

Cognitive tasks: For the Reversals, the number of trials and the number of errors to criterion were analyzed with repeated measures ANOVA with Group (control, E₂) as between subject factor and Reversal Number (1, 2, 3) as within subject factor. For the Delayed Response, a repeated measures ANOVA was performed on the percentage of correct responses with Group as a between subject factor and Delays (0, 1, 3, 6, and 10s) as a repeated factor. For the Detour Reaching task, the number of reaches and latencies to get the reward were submitted to a repeated measure ANOVA with Group as a between subject factor and Orientation (Centre, Left, Right) and Session (1-10) as repeated factors. For the acquisition of the DMP, the number of trials and number of errors were compared for each group using a one-tailed independent t-test. A one tailed t-test was used because of the prediction that E₂ would enhance the acquisition of this spatial task, based on rodent data (65, 69, 70). For the analysis of spatial pattern separation, 3 blocks of 12 trials were considered, so as to capture the beginning, middle and last phases of learning (i.e., first 12 trials, median 12 trials and last 12 trials) in monkeys that successfully learned the task. ANOVAs with Block (First, Median and Last) as within subject factor and Group as between subject factor were conducted on percent accuracy for each condition (Close, Medium, Far) considered separately.

To supplement the ANOVAs, a standardized measure of effect size (Cohen's d for t-tests and f^2 for ANOVAs) was calculated using G*Power 3.1 to estimate the size of the observed differences between treatment groups (71). Cohen's $d = 0.20$ represents small differences, $d = 0.50$ represents medium differences, and $d = 0.80$ represents large differences. Cohen's $f^2 = 0.02$ represents small differences, $d = 0.15$ represents medium differences, and $f^2 = 0.35$ represents large differences.

RESULTS

Oestrogen measures

Due to unforeseen problems with the urine assays, oestrogen measures were determined from 2 plasma samples collected at weeks 18 and 19 of the experiment, that is, 5 and 4 weeks prior to the end of the study. As can be seen in Fig. 2, OVX females implanted with E₂ capsules exhibited higher plasma oestrogen levels (mean = 360.83 + 24.21 pg/ml) than

females implanted with empty capsules (mean = 209.58 + 50.02 pg/ml; $t(10) = -2.072$, $p < 0.05$). Yet, one control monkey had unexpectedly high oestrogen values (> 400 ng/ml) that were 2 standard deviations above the mean of the group. This outlier was excluded from all subsequent analyses. Re-analysis of the data without this individual indicated that plasma oestrogen levels achieved by E₂-treated monkeys were significantly higher than those of the control monkeys (mean = 161.20 + 15.356; $t(9) = -6.60$, $p < 0.001$).

Behavioural Data

No difference was found between the E₂ and control groups for either the frequency of all affiliative behaviours ($t(9) = -0.31$, $p = 0.76$), the frequency of social contacts initiated by the female ($t(9) = -1.09$, $p = 0.30$) or the frequency of social contacts received by the female ($t(9) = 0.17$, $p = 0.87$). In addition, the frequency of these behaviours did not significantly differ as a function of Time (first 7 days, last 7 days) or Time $\times$ Treatment (all $ps > 0.05$); see Fig. 3.

Object Reversals

The number of trials and errors to reach criterion on the initial object discrimination can be seen in Fig. 4. Preoperatively, the monkeys reached the learning criterion in an average of 47.63 + 7.09 trials and 19.54 + 2.74 errors (last 20 trials omitted). Reversal tests were administered post-ovariectomy and treatment. A repeated measures ANOVA indicated that the number of trials to reach criterion did not significantly vary as a function of Reversal Number ($F(2,18) = 1.74$, $p = 0.20$; effect size $f^2 = 0.44$), Group ($F(1, 9) = 2.40$, $p = .16$; $f^2 = 0.51$) or Reversal Number $\times$ Group ($F(2, 18) = 1.81$, $p = 0.19$; $f^2 = 0.50$). Similarly, the main effects of Reversal Number ($F(2,18) = 0.73$, $p = 0.50$; $f^2 = 0.28$) and Group ($F(1, 9) = 0.15$, $p = .22$, $f^2 = 0.53$) did not reach significance for the number of errors. The Reversal Number $\times$ Group interaction ($F(2, 18) = 2.57$, $p = 0.10$; $f^2 = 0.53$) failed to reach significance. Despite the lack of statistical significance, the magnitude of the effects was large for each of these comparisons. Based on these large effect sizes, we conducted exploratory analyses with independent Student *t*-tests to compare the number of trials and errors committed by each group in each reversal. This analysis revealed that the number of trials needed to reach criterion ($t(9) = -3.20$, $p < .02$) as well as the number of errors committed ($t(9) = -2.60$, $p < .05$) were significantly higher in the E₂ compared to the control group for the second reversal. The other comparisons were not significant. The ANOVAs on the number of perseverative errors, and errors when the animals performed at chance or during the learning stage indicated that these measures were not significantly affected by Reversal number, Group or Reversal $\times$ Group interaction (all $ps > .05$). However, linear contrasts examining the linear trends in the mean number of perseverative errors as a function of reversal number revealed a significant Reversal $\times$ Group interaction ($F(1, 9) = 6.13$, $p < .05$). As can be seen in Fig. 4, monkeys in the E₂ group had an increasing number of perseverative errors from Reversals 1 to 3, while the number of perseverative errors decreased during the same period in the control monkeys.

Delayed Response

The number of trials to reach criterion and the number of errors for each delay when learning the Delayed Response pre-surgery can be seen in Table 2. The memory aspect of

the task was challenging and yielded important individual differences: the number of trials required to reach learning criterion increased from an average of 21 trials for the 0 delay condition, to 50 trials and 76 trials when delays of 1s and 3s were implemented, respectively. While acquisition of the 6s delay was somewhat faster (44 trials), learning the 10s delay required an average of 75 trials, with individuals differing greatly in their ability to perform the task with long delays. Following ovariectomy and treatment, monkeys were tested on 100 trials of the Delayed Response with mixed delays. The ANOVA revealed a significant effect of Delay on the percentage of correct responses ($F(4, 36) = 19.57$, $p < 0.001$; $f^2 = 1.47$), a marginal effect of Group ($F(1, 9) = 3.90$, $p = 0.08$; $f^2 = 0.66$) and a non significant Delay $\times$ Group interaction ($F(4, 36) = 0.28$, $p = 0.13$; $f^2 = 0.44$; Fig. 5). As can be seen in Fig.5, performance decreased significantly as a function of increasing delays and E_2 -treated monkeys tended to have lower scores than control monkeys on the task.

Detour Reaching

Prior to ovariectomy, monkeys were trained on the task with an opaque box. Each monkey was required to perform 2 successful reaches towards each orientation (centre, left, right). This step was readily performed by all animals. Following ovariectomy and treatment, monkeys were tested on 10 sessions (120 trials) of the Detour reaching with a transparent box. The ANOVA revealed a significant effect of orientation on the number of reaching errors ($F(2,18) = 36.25$, $p < .001$, $f^2 = 2.0$) and on the latencies to get the reward ($F(2,18) = 6.44$, $p < .01$, $f^2 = 0.84$), indicating that monkeys committed less errors and were faster for the central than the left or right orientations, with no significant difference between the left and right orientations. A significant effect of Session on the number of reaching errors ($F(9, 81) = 9.90$, $p < .001$, $f^2 = 1.04$) and latencies ($F(9, 81) = 3.16$, $p < .005$, $f^2 = 0.59$) also indicated that performance became more accurate and faster with practice (see Fig. 6). The effect of Group was not significant for either the number of reaching errors $F(1, 9) = 0.34$, $p = 0.86$, $f^2 = 0.06$ or latencies $F(1, 9) = 0.31$, $p = 0.59$, $f^2 = 0.19$. The interaction between Orientation and Group was not significant for either the number of reaches errors ($F(2,18) = 0.042$, $p = 0.96$, $f^2 = 0.07$) or the latencies $F(2,18) = 0.91$, $p = 0.42$, $f^2 = 0.31$). Similarly, the interaction between Session and Group was not significant for either the reaches errors ($F(9, 81) = 0.55$, $p = 0.83$, $f^2 = 0.25$) or latencies $F(9, 81) = 1.03$, $p = 0.42$, $f^2 = 0.34$).

DMP acquisition

After monkeys completed testing on the 3 tasks described above, they were administered the DMP with 4 locations to test the hypothesis that E_2 would improve acquisition of a spatial task. All 6 monkeys in the E_2 group but only 3/5 monkeys in the control group learned the task within 350 trials. In addition, monkeys in the E_2 group learned the task in an average of 149 trials + 35.87 vs. 232 trials + 49.86 for monkeys in the control group. This difference failed to reach significance $t(9) = -1.38$, $p = .10$, one-tailed; effect size $d = 0.83$). Similarly, the E_2 group tended to make less errors (64.17 + 15) than the control group (110 + 29.61; $t(9) = 3.12$, $p = .09$; one-tailed, $d = 0.86$; Fig 7) but the difference did not reach significance. The control monkeys not learning the task ($n=2$) were given the maximum number of trials and errors they reached by the time the study ended. As these numbers could have been considerably larger had we continued testing until monkeys learned the task, these results are highly conservative.

We also investigated whether learning performance varied as a function of spatial pattern separation. For this analysis, the percentage accuracy in the first, median and last blocks of 12 trials was examined as a function of the relative location of the two tokens (Close, Medium or Far) for the 9 monkeys that had successfully learned the task. For the Close pattern, the effect of Block was significant ($F(2, 14)=9.26, p<.01$), indicating better performance as testing progressed. The effect of Treatment was of marginal significance ($F(1,7)=3.80, p<.10$) but the interaction between Block and Treatment was not significant ($F(2,14)=0.31, ns$). For the Medium pattern, a significant effect of Block was observed ($F(2,14)=4.37, p<.05$) but there was no effect of Treatment ($F(1,7)=.09, ns$) or interaction with Block ($F(2,14)=0.24, ns$). For the Far pattern, the effects of Block ($F(2, 14)=17.07, p<.001$) and Block $\times$ Treatment ($F(2,14)=3.81, p<.05$) were significant. As can be seen in Fig. 7, the interaction indicated that while control monkeys were better than E₂-treated monkeys for the first block of trials, the reverse was true when the last block of trials was considered.

DISCUSSION

This is the first report using the common marmoset as a primate model for studying the effects of oestrogens on cognitive function. The study shows that adult female marmosets can be trained on a battery of tasks commonly used in the cognitive aging literature, with performance levels roughly equivalent to those of rhesus monkeys in the same tasks. These findings support the use of the marmoset as an alternative primate model for studying human cognitive aging (see 22, 72). With regard to oestrogens effects, we found evidence of impaired Object Reversal and Delayed Response performance in the E₂-treated group relative to the control group. Specifically, E₂-treated monkeys needed more trials and committed more errors than control monkeys in the second reversal. In addition, perseverative responding increased from reversals 1-3 in the E₂ group, while it decreased in the control monkeys. The effect of E₂ treatment for the Delayed Response task was only significant at the .08 level of confidence and needs to be interpreted with caution. Nevertheless, the large effect size (Cohen's $f^2 = 0.66$) suggests that the detrimental effect of treatment in this task was meaningful and that our sample size was likely too small to detect group differences at the .05 level. Finally, the results suggest potentially enhancing effects of E₂ treatment in the acquisition of the DMP, but additional studies are needed to confirm this finding. All 6 monkeys in the E₂ group, but only 3 monkeys in the control group acquired the task in 350 trials or less. Second, the trials and the number errors to reach criterion tended to be lower in the E₂ group relative to the control group. Although these differences did not reach statistical significance, the effect sizes were large. No evidence for group differences on the Detour Reaching task, a task of motor inhibition, or in affiliative behaviours was found.

The results on the two prefrontal tasks are consistent with the findings of several rodent studies. Negative effects of E₂ replacement have been reported in aged OVX rats tested on Object reversals (73) and across age groups (young, middle-aged and aged) in OVX female rats tested on another prefrontally-mediated task, the Delayed Spatial Alternation task (74). The Object Reversals and the Delayed Response tasks assess different aspects of executive function, with the Reversals targeting cognitive flexibility, and the Delayed Response task assessing the ability to maintain spatial information in short-term memory. For the Object

Reversal task, it is possible that E2 increased attention to response/reward contingencies during the 1st reversal, thus impairing behavioural flexibility during the 2nd reversal. Indeed, E2 has been shown to increase attention in previous studies in female OVX macaques (75-77). In this regard, future studies should examine differences in the rate of task acquisition (initial discrimination) in E2 vs. control OVX monkeys, as increased attention to response/reward contingencies would be expected to facilitate acquisition in the E2 group. An interpretation solely based on attention cannot account for the results from the Delayed Response task, however, since enhanced attention to the baited stimulus would be predicted to enhance performance in this task. Thus specific deficits in the ability to maintain the information in memory would be more likely in this task.

Performance on these two tasks has been shown to be modulated by dopamine (DA). For example, an early study in marmosets (78) showed that amphetamine (a DA agonist) impaired reversal performance by increasing perseverative responding, an effect that could be prevented by pretreatment with haloperidol, a DA antagonist. In addition, later marmoset studies showed that DA depletion in the caudate nucleus (79) produced impairments in the Reversals, although serotonin was also involved at the level of the orbitofrontal cortex (79, 80). Robust evidence indicates that performance on the Delayed Response task is also dependent on dopaminergic signaling (57, 81). Interestingly, the relationship of DA to working memory performance follows an inverted U shape, with suboptimal or excessive DA levels impairing working memory (82-84).

Oestrogens have been shown to enhance the activity of dopaminergic neurones (85), increase dopaminergic innervation in the prefrontal cortex (86) and protect dopaminergic neurones from a variety of insults (87). In addition, studies in rats (88, 89) and humans (90) have demonstrated clear interactions between the dopaminergic system, oestrogens and working memory performance. Specifically, Shansky et al. (2004) (88) found that T-maze performance was more severely impaired by the administration of a dopaminergic drug in female rats tested in pro-estrus (high oestrogen levels) relative to females tested at other phases of the cycle, a phenomenon that could be replicated in OVX females replaced with E₂ (89). In the women study, young women genotyped for the catechol-O-methyltransferase (COMT) Val158Met polymorphism were tested on a working memory task during the menses and the late follicular phase of the cycle. Working memory performance was modulated both by cycle phase and baseline prefrontal DA in such a way that women with high COMT activity (indexing low baseline prefrontal DA) had maximal performance during the late follicular phase, when E₂ levels are high, while women with high baseline prefrontal DA performed best during the menses, when E₂ levels are low (90). It can be concluded from these studies that E₂ may have beneficial or detrimental effects on working memory depending on the dopaminergic milieu.

Based on these findings and the observations of the present study, we speculate that oestrogens may impair Reversal learning and Delayed Response performance via enhancement of dopaminergic activity in the PFC. This does not exclude the intervention of other neurotransmitter systems. Several lines of evidence suggest that the serotonergic system could also be involved. First, serotonin in the orbitofrontal cortex is necessary for Reversal learning performance in marmosets (80). Second, oestrogens increase serotonin

levels in the rat prefrontal cortex (91). Third, two imaging studies in postmenopausal women suggest that oestrogens increase serotonin binding in the prefrontal cortex (92) and brain activation during working memory performance (93). Finally, elevated serotonin release, as assessed with microdialysis, has been associated with increased impulsive responding in the 5-choice serial reaction time task in rats (94). Thus, it is possible that in addition to DA, E₂ treatment increased serotonin in the PFC. Future studies are needed to examine the specific contribution of each neurotransmitter in relation to the oestrogen milieu and working memory performance.

Our findings showing E₂-induced deficits in PFC-dependent tasks are consistent with the rat studies mentioned above, but contrast with the results of several studies in women and female macaques. Positive effects of oestrogen use have been found in postmenopausal women performing working memory tasks (95, 96). Similarly, two macaque studies found evidence for E₂ enhancement of performance on PFC-mediated tasks. In one study, E₂-treated middle-aged female macaques had enhanced Delayed Response performance relative to vehicle-treated animals (54). In another study, aged OVX monkeys treated with E₂ had enhanced set shifting abilities in a monkey version of the Wisconsin Card Sort Test compared to placebo-treated animals (77). Yet, no effect of E₂ on a similar task in long-term OVX females have also been reported (97). The basis for these discrepant findings is unclear at this point, but could potentially be due to differences in treatment regimen. Indeed the two macaque studies reporting positive effects of E₂ on PFC tasks used either a cyclic regimen (E₂ cypionate injection every 3 weeks) which produced a peak in E₂ declining throughout 3 weeks, or chronic replacement (17 β -E₂ in Silastic capsules) combined with one injection of E₂ valerate on day 12 which produced a peak of E₂ at that time. In contrast, Lacreuse et al., (2004) (97) administered oral ethinyl E₂ at constant levels for 28 days. It has been proposed that administration regimens that produce fluctuating levels of E₂, as opposed to stable E₂ levels, may be necessary for enhancing cognition, at least in the prefrontal cortex (98). This possibility needs to be tested in future studies.

The findings regarding the acquisition of the DMP are difficult to interpret in the absence of statistical significance. Learning of this task has consistently been shown to be improved by oestrogens in several rat studies (65, 69, 70) but the neural circuitry underlying task performance remains unclear, with both the hippocampus and PFC contributing to the task. Since spatial pattern separation is thought to engage the hippocampus, we had hypothesised that E₂-treated monkeys would be outperform control monkeys for trials in which the two tokens were in close proximity (Close trials), as opposed to far apart (Far condition). Our analysis of performance accuracy as a function of spatial pattern separation does not provide support for this hypothesis. Yet, based on the large effect sizes we observed and the inability of 2 control monkeys to learn the task within 350 trials, our results suggest a potential positive effect of E₂ treatment on DMP learning in marmosets. These results can only be considered preliminary at this point and will need to be validated in future studies with a larger sample size.

There was no evidence for oestrogen effects on Detour Reaching or affiliative behaviours. At first glance, the lack of E₂ influence on Detour Reaching may be surprising since this task targets inhibitory control, a function dependent on the prefrontal cortex (58). However,

our task was likely too easy to be sensitive to oestrogens, as the number of errors decreased very rapidly during the course of testing. Indeed, the interaction between DA and oestrogens is mainly observed on tasks requiring high cognitive control (90).

As far as behaviour is concerned, we were predicting an increase in affiliative behaviours in E₂-treated monkeys relative to the control group. However, enhancing effects of E₂ on female marmoset proceptive behaviour has been found in some (99, 100) but not all studies (66). Their behaviour may have been influenced by that of their male partners, who were castrated. Indeed, hyporesponsiveness of males has been shown to reduce the influence of E₂ on proceptive behaviours in OVX marmosets (101). In addition, chronic replacement with constant E₂ levels may reduce the effectiveness of E₂ in stimulating sexual behaviours (66).

In conclusion, this study demonstrates that the female marmoset is a useful new primate model to examine the effects of oestrogens on cognitive function. The findings suggest that treatment with 17 β -E₂ differentially influences selective cognitive domains in adult OVX female marmosets. Tasks involving prefrontal function were impaired by treatment, perhaps due to E₂-mediated stimulation of the dopaminergic and/or serotonergic system in the PFC. E₂ effects on the DMP task, which involves the hippocampus, were suggestive of improved learning, but additional studies with a larger sample size are needed to confirm this observation. It would be useful to extend the study to younger as well as older subjects in order to interpret the findings in the context of the aging process. Because marmosets can be studied longitudinally throughout their entire lifespan, they will be particularly valuable in advancing our understanding of the long-term effects of oestrogen exposure on the brain and cognition.

Acknowledgments

This research was supported by NIH grant # MH091492 to Agnès Lacreuse. We are very grateful to Evan Palamara, Kelly Schatz, Eva Wilbar and Stephen Ferrigno for their help with data collection. We thank Drs. David Abbott and Nafissa Ismail for their input on the Silastic implant procedures and Dr. Pamela Tannenbaum for helpful discussions. Many thanks to Dr. Margaret Piwonka and the UMass veterinary staff, Animal Care Staff and Shop staff for their expert assistance.

REFERENCES

1. Salthouse TA. Selective review of cognitive aging. *J Int Neuropsychol Soc.* 2010; 16(5):754–60. [PubMed: 20673381]
2. Park, D.; Schwartz, N. *Cognitive aging: a primer.* Psychology Press; Philadelphia, PA: 2000.
3. Gure TR, Langa KM, Fisher GG, Piette JD, Plassman BL. Functional limitations in older adults who have cognitive impairment without dementia. *J Geriatr Psychiatry Neurol.* 2013; 26(2):78–85. [PubMed: 23559664]
4. Kinsella, K.; Wan, H. US Census Bureau, International Population Reports, An Aging World. US Government Printing Office; Washington, DC: 2009.
5. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J Clin Endocrinol Metab.* 2001; 86(2):724–31. [PubMed: 11158037]
6. Burger HG. The endocrinology of the menopause. *J Steroid Biochem Mol Biol.* 1999; 69(1-6):31–5. [PubMed: 10418978]

7. Sherwin BB. Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. *Psychoneuroendocrinology*. 1988;13:345–57.
8. McEwen BS, Alves SE, Bulloch K, Weiland NG. Ovarian steroids and the brain: implications for cognition and aging. *Neurology*. 1997; 48(Suppl 7):S8–S15. [PubMed: 9153161]
9. Halbreich U, Lumley LA, Palter S, Manning C, Gengo F, Joe SH. Possible acceleration of age effects on cognition following menopause. *J Psychiatr Res*. 1995; 29(3):153–63. [PubMed: 7473292]
10. Sherwin BB. Estrogen and cognitive functioning in women. *Endocr Rev*. 2003; 24(2):133–51. [PubMed: 12700177]
11. Gibbs RB, Gabor R. Estrogen and cognition: applying preclinical findings to clinical perspectives. *J Neurosci Res*. 2003; 74(5):637–43. [PubMed: 14635215]
12. Rossouw JE. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *J Am Med Assoc*. 2002; 288:321–33.
13. Rapp SR, Espeland MA, Shumaker SA, Henderson VW, Brunner RL, Manson JE, Gass MLS, Stefanick ML, Lane DS, Hays J, Johnson KC, Coker LH, Dailey M, Bowen D. Effect of Estrogen Plus Progestin on Global Cognitive Function in Postmenopausal Women: The Women's Health Initiative Memory Study: A Randomized Controlled Trial. *J Am Med Assoc*. 2003; 289(20):2663–72.
14. Shumaker SA, Legault C, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones BN III, Assaf AR, Jackson RD, Morley Kotchen J, Wassertheil-Smoller S, Wactawski-Wende J. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: The Women's Health Initiative Memory Study: A Randomized Controlled Trial. *J Am Med Assoc*. 2003; 289(20):2651–62.
15. Maki PM, Henderson VW. Hormone therapy, dementia, and cognition: the Women's Health Initiative 10 years on. *Climacteric*. 2012; 15(3):256–62. [PubMed: 22612612]
16. Frick KM. Building a better hormone therapy? How understanding the rapid effects of sex steroid hormones could lead to new therapeutics for age-related memory decline. *Behav Neurosci*. 2012; 126(1):29–53. [PubMed: 22289043]
17. Brinton DR. Minireview: Translational Animal Models of Human Menopause: Challenges and Emerging Opportunities. *Endocrinology*. 2012; 153(8):3571–8. [PubMed: 22778227]
18. Capitanio JP, Emborg ME. Contributions of non-human primates to neuroscience research. *The Lancet*. 2008; 371(9618):1126–35.
19. Lacreuse A. Effects of ovarian hormones on cognitive function in nonhuman primates. *Neuroscience*. 2006; 138(3):859–67. [PubMed: 16310311]
20. Voytko ML, Tinkler GP, Browne C, Tobin JR. Neuroprotective effects of estrogen therapy for cognitive and neurobiological profiles of monkey models of menopause. *Am J Primatol*. 2009; 71(9):794–801. [PubMed: 19475542]
21. Morrison JH, Brinton RD, Schmidt PJ, Gore AC. Estrogen, Menopause, and the Aging Brain: How Basic Neuroscience Can Inform Hormone Therapy in Women. *J Neurosci*. 2006; 26(41):10332–48. [PubMed: 17035515]
22. Lacreuse, A.; Herndon, JG. Nonhuman primate models of cognitive aging. In: Bizon, JL.; Woods, CE., editors. *Animals Models of Human Cognitive Aging*. Humana Press; New York, NY: 2009. p. 29-58.
23. Tardif SD, Mansfield KG, Ratnam R, Ross CN, Ziegler TE. The marmoset as a model of aging and age-related diseases. *ILAR J*. 2011; 52(1):54–65. [PubMed: 21411858]
24. Austad SN, Fischer KE. The development of small primate models for aging research. *ILAR J*. 2011; 52(1):78–88. [PubMed: 21411860]
25. Ross CN, Davis K, Dobek G, Tardif SD. Aging Phenotypes of Common Marmosets (*Callithrix jacchus*). *Journal of Aging Research*. 2012; 2012
26. Tardif SD, Smucny DA, Abbott DH, Mansfield K, Schultz-Darken N, Yamamoto ME. Reproduction in captive common marmosets (*Callithrix jacchus*). *Comp Med*. 2003; 53(4):364–8. [PubMed: 14524412]

27. Abbott DH, Barnett DK, Colman RJ, Yamamoto ME, Schultz-Darken NJ. Aspects of common marmoset basic biology and life history important for biomedical research. *Comp Med*. 2003; 53(4):339–50. [PubMed: 14524409]
28. Nishijima K, Saitoh R, Tanaka S, Ohsato-Suzuki M, Ohno T, Kitajima S. Life span of common marmoset (*Callithrix jacchus*) at CLEA Japan breeding colony. *Biogerontology*. 2012; 13(4):439–43. [PubMed: 22752736]
29. Leuner B, Kozorovitskiy Y, Gross CG, Gould E. Diminished adult neurogenesis in the marmoset brain precedes old age. *Proc Natl Acad Sci*. 2007; 104(43):17169–73. [PubMed: 17940008]
30. Geula C, Nagykeri N, Wu CK. Amyloid-beta deposits in the cerebral cortex of the aged common marmoset (*Callithrix jacchus*): incidence and chemical composition. *Acta Neuropathol (Berl)*. 2002; 103(1):48–58. [PubMed: 11837747]
31. Sasaki E, Suemizu H, Shimada A, Hanazawa K, Oiwa R, Kamioka M, Tomioka I, Sotomaru Y, Hirakawa R, Eto T, Shiozawa S, Maeda T, Ito M, Ito R, Kito C, Yagihashi C, Kawai K, Miyoshi H, Tanioka Y, Tamaoki N, Habu S, Okano H, Nomura T. Generation of transgenic non-human primates with germline transmission. *Nature*. 2009; 459(7246):523–7. [PubMed: 19478777]
32. Stephan, H.; Baron, G.; Schwerdtfeger, WK. The brain of the common marmoset (*Callithrix jacchus*). Springer-Verlag; 1980.
33. Rilling JK. Human and nonhuman primate brains: are they allometrically scaled versions of the same design? *Evol Anthropol*. 2006; (15):65–77.
34. Chaplin TA, Yu H-H, Soares JGM, Gattass R, Rosa MGP. A Conserved Pattern of Differential Expansion of Cortical Areas in Simian Primates. *J Neurosci*. 2013; 33(38):15120–5. [PubMed: 24048842]
35. Hikishima K, Quallo MM, Komaki Y, Yamada M, Kawai K, Momoshima S, Okano HJ, Sasaki E, Tamaoki N, Lemon RN, Iriki A, Okano H. Population-averaged standard template brain atlas for the common marmoset (*Callithrix jacchus*). *NeuroImage*. 2011; 54(4):2741–9. [PubMed: 21044887]
36. Newman JD, Kenkel WM, Aronoff EC, Bock NA, Zametkin MR, Silva AC. A combined histological and MRI brain atlas of the common marmoset monkey, *Callithrix jacchus*. *Brain Res Rev*. 2009; 62(1):1–18. [PubMed: 19744521]
37. Brevard ME, Meyer JS, Harder JA, Ferris CF. Imaging brain activity in conscious monkeys following oral MDMA (“ecstasy”). *Magn Res Imaging*. 2006; 24(6):707–14.
38. Ferris CF, Snowdon CT, King JA, Duong TQ, Ziegler TE, Ugurbil K, Ludwig R, Schultz-Darken NJ, Wu Z, Olson DP, Sullivan JM Jr, Tannenbaum PL, Vaughan JT. Functional imaging of brain activity in conscious monkeys responding to sexually arousing cues. *Neuroreport*. 2001; 12(10):2231–6. [PubMed: 11447340]
39. Belcher AM, Yen CC, Stepp H, Gu H, Lu H, Yang Y, Silva AC, Stein EA. Large-Scale Brain Networks in the Awake, Truly Resting Marmoset Monkey. *J Neurosci*. 2013; 33(42):16796–804. [PubMed: 24133280]
40. Miles RC. Delayed-response learning in the marmoset and the macaque. *J Comp Physiol Psychol*. 1957:50352–5.
41. Spinelli S, Pennanen L, Dettling AC, Feldon J, Higgins GA, Pryce CR. Performance of the marmoset monkey on computerized tasks of attention and working memory. *Cogn Brain Res*. 2004; 19(2):123–37.
42. Takemoto A, Izumi A, Miwa M, Nakamura K. Development of a compact and general-purpose experimental apparatus with a touch-sensitive screen for use in evaluating cognitive functions in common marmosets. *J Neurosci Methods*. 2011; 199(1):82–6. [PubMed: 21549751]
43. Strasser A, Burkart JM. Can we measure brain efficiency? an empirical test with common marmosets (*Callithrix jacchus*). *Brain Behav Evol*. 2012; 80(1):26–40. [PubMed: 22846401]
44. Hearn, JP. The common marmoset. In: Hearn, JP., editor. *Reproduction in New World Primates*. MTP press; Lancaster: 1983. p. 181-216.
45. Tardif SD, Araujo A, Arruda MF, French JA, Sousa MB, Yamamoto ME. Reproduction and aging in marmosets and tamarins. *Interdiscip Top Gerontol*. 2008:3629–48.
46. Gilardi KV, Shideler SE, Valverde CR, Roberts JA, Lasley BL. Characterization of the onset of menopause in the rhesus macaque. *Biol Reprod*. 1997:57335–40.

47. Stevenson MF, Poole TB. An ethogram of the common marmoset (*Calithrix jacchus jacchus*): General behavioural repertoire. *Animal Behav.* 1976; 24(2):428–51.
48. Dias R, Robbins TW, Roberts AC. Dissociation in prefrontal cortex of affective and attentional shifts. *Nature.* 1996; 380(6569):69–72. [PubMed: 8598908]
49. Clarke HF, Robbins TW, Roberts AC. Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. *J Neurosci.* 2008; 28(43):10972–82. [PubMed: 18945905]
50. Rygula R, Walker SC, Clarke HF, Robbins TW, Roberts AC. Differential contributions of the primate ventrolateral prefrontal and orbitofrontal cortex to serial reversal learning. *J Neurosci.* 2010; 30(43):14552–9. [PubMed: 20980613]
51. Ridley RM, Baker HF, Mills DA, Green ME, Cummings RM. Topographical memory impairments after unilateral lesions of the anterior thalamus and contralateral inferotemporal cortex. *Neuropsychologia.* 2004; 42(9):1178–91. [PubMed: 15178170]
52. Lai ZC, Moss MB, Killiany RJ, Rosene DL, Herndon JG. Executive system dysfunction in the aged monkey: spatial and object reversal learning. *Neurobiol Aging.* 1995; 16(6):947–54. [PubMed: 8622786]
53. Goldman PS, Rosvold HE. Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. *Exp Neurol.* 1970:27291–304.
54. Rapp PR, Morrison JH, Roberts JA. Cyclic estrogen replacement improves cognitive function in aged ovariectomized rhesus monkeys. *J Neurosci.* 2003; 23(13):5708–14. [PubMed: 12843274]
55. Voytko ML, Higgs CJ, Murray R. Differential effects on visual and spatial recognition memory of a novel hormone therapy regimen of estrogen alone or combined with progesterone in older surgically menopausal monkeys. *Neuroscience.* 2008; 154(4):1205–17. [PubMed: 18554815]
56. Lacreuse A, Wilson ME, Herndon JG. Estradiol, but not raloxifene, improves aspects of spatial working memory in aged ovariectomized rhesus monkeys. *Neurobiol Aging.* 2002; 23(4):589–600. [PubMed: 12009508]
57. Collins P, Wilkinson LS, Everitt BJ, Robbins TW, Roberts AC. The effect of dopamine depletion from the caudate nucleus of the common marmoset (*Callithrix jacchus*) on tests of prefrontal cognitive function. *Behav Neurosci.* 2000; 114(1):3–17. [PubMed: 10718258]
58. Wallis JD, Dias R, Robbins TW, Roberts AC. Dissociable contributions of the orbitofrontal and lateral prefrontal cortex of the marmoset to performance on a detour reaching task. *Eur J Neurosci.* 2001; 13(9):1797–808. [PubMed: 11359531]
59. Aggleton JP, Keith AB, Rawlins JN, Hunt PR, Sahgal A. Removal of the hippocampus and transection of the fornix produce comparable deficits on delayed non-matching to position by rats. *Behav Brain Res.* 1992; 52(1):61–71. [PubMed: 1472288]
60. Hampson RE, Jarrard LE, Deadwyler SA. Effects of ibotenate hippocampal and extrahippocampal destruction on delayed-match and -nonmatch-to-sample behavior in rats. *J Neurosci.* 1999; 19(4):1492–507. [PubMed: 9952425]
61. Dudchenko PA, Talpos J, Young J, Baxter MG. Animal models of working memory: A review of tasks that might be used in screening drug treatments for the memory impairments found in schizophrenia. *Neurosci Biobehav Rev.* 2013; 37(9):211–2124.
62. McAllister KA, Saksida LM, Bussey TJ. Dissociation between memory retention across a delay and pattern separation following medial prefrontal cortex lesions in the touchscreen TUNL task. *Neurobiol Learn Mem.* 2013:101120–6.
63. Talpos JC, McTighe SM, Dias R, Saksida LM, Bussey TJ. Trial-unique, delayed nonmatching-to-location (TUNL): a novel, highly hippocampus-dependent automated touchscreen test of location memory and pattern separation. *Neurobiol Learn Mem.* 2010; 94(3):341–52. [PubMed: 20692356]
64. Kesner RP, Rogers J. An analysis of independence and interactions of brain substrates that subserve multiple attributes, memory systems, and underlying processes. *Neurobiol Learn Mem.* 2004; 82(3):199–215. [PubMed: 15464404]
65. Gibbs RB. Estradiol enhances DMP acquisition via a mechanism not mediated by turning strategy but which requires intact basal forebrain cholinergic projections. *Horm Behav.* 2007; 52(3):352–9. [PubMed: 17583704]

66. Barnett DK, Bunnell TM, Millar RP, Abbott DH. Gonadotropin-releasing hormone II stimulates female sexual behavior in marmoset monkeys. *Endocrinology*. 2006; 147(1):615–23. [PubMed: 16179411]
67. Saltzman W, Pick RR, Salper OJ, Liedl KJ, Abbott DH. Onset of plural cooperative breeding in common marmoset families following replacement of the breeding male. *Anim Behav*. 2004; 68(1):59–73.
68. Saltzman W, Schultz-Darken NJ, Wegner FH, Wittwer DJ, Abbott DH. Suppression of cortisol levels in subordinate female marmosets: reproductive and social contributions. *Horm Behav*. 1998; 33(1):58–74. [PubMed: 9571014]
69. Gibbs RB. Estrogen replacement enhances acquisition of a spatial memory task and reduces deficits associated with hippocampal muscarinic receptor inhibition. *Horm Behav*. 1999; 36(3):222–33. [PubMed: 10603286]
70. Hammond R, Mauk R, Ninaci D, Nelson D, Gibbs RB. Chronic treatment with estrogen receptor agonists restores acquisition of a spatial learning task in young ovariectomized rats. *Horm Behav*. 2009; 56(3):309–14. [PubMed: 19560466]
71. Cohen, J. Statistical power analysis in behavioral research. L. Erlbaum Associates; Hillsdale, NJ: 1988.
72. Lacreuse A. Neurocognitive effects of hormonal replacement therapy: key advances from nonhuman primate research. *J Primatol*. 2012; 1(e108)
73. Gibbs RB, Chipman AM, Hammond R, Nelson D. Galanthamine plus estradiol treatment enhances cognitive performance in aged ovariectomized rats. *Horm Behav*. 2011; 60(5):607–16. [PubMed: 21889940]
74. Wang VC, Neese SL, Korol DL, Schantz SL. Chronic estradiol replacement impairs performance on an operant delayed spatial alternation task in young, middle-aged, and old rats. *Horm Behav*. 2009; 56(4):382–90. [PubMed: 19631212]
75. Voytko ML, Kitt CA, Price DL. Cholinergic immunoreactive fibers in monkey anterior temporal cortex. *Cereb Cortex*. 1992; 2(1):48–55. [PubMed: 1633407]
76. Voytko ML. Estrogen and the cholinergic system modulate visuospatial attention in monkeys (*Macaca fascicularis*). *Behav Neurosci*. 2002;116187–97.
77. Voytko ML, Murray R, Higgs CJ. Executive function and attention are preserved in older surgically menopausal monkeys receiving estrogen or estrogen plus progesterone. *J Neurosci*. 2009; 29(33):10362–70. [PubMed: 19692611]
78. Ridley RM, Haystead TA, Baker HF. An analysis of visual object reversal learning in the marmoset after amphetamine and haloperidol. *Pharmacol Biochem Behav*. 1981; 14(3):345–51. [PubMed: 6785766]
79. Clarke HF, Hill GJ, Robbins TW, Roberts AC. Dopamine, but not serotonin, regulates reversal learning in the marmoset caudate nucleus. *J Neurosci*. 2011; 31(11):4290–7. [PubMed: 21411670]
80. Clarke HF, Dalley JW, Crofts HS, Robbins TW, Roberts AC. Cognitive inflexibility after prefrontal serotonin depletion. *Science*. 2004; 304(5672):878–80. [PubMed: 15131308]
81. Sawaguchi T, Goldman-Rakic PS. D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science*. 1991; 251(4996):947–50. [PubMed: 1825731]
82. Cai JX, Arnsten AF. Dose-dependent effects of the dopamine D1 receptor agonists A77636 or SKF81297 on spatial working memory in aged monkeys. *J Pharmacol Exp Ther*. 1997; 283(1):183–9. [PubMed: 9336323]
83. Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ, Robbins TW. Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *J Neurosci*. 2000; 20(3):1208–15. [PubMed: 10648725]
84. Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. *Nat Neurosci*. 2007; 10(3):376–84. [PubMed: 17277774]
85. Becker JB. Oestrogen effects on dopaminergic function in striatum. *Novartis Found Symp*. 2000;230134–45.

86. Kritzer MF, Creutz LM. Region and sex differences in constituent dopamine neurons and immunoreactivity for intracellular estrogen and androgen receptors in mesocortical projections in rats. *J Neurosci.* 2008; 28(38):9525–35. [PubMed: 18799684]
87. Bourque M, Dluzen DE, Di Paolo T. Neuroprotective actions of sex steroids in Parkinson's disease. *Front Neuroendocrinol.* 2009; 30(2):142–57. [PubMed: 19410597]
88. Shansky RM, Glavis-Bloom C, Lerman D, McRae P, Benson C, Miller K, Cosand L, Horvath TL, Arnsten AF. Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. *Mol Psychiatry.* 2004; 9(5):531–8. [PubMed: 14569273]
89. Shansky RM, Bender G, Arnsten AF. Estrogen prevents norepinephrine alpha-2a receptor reversal of stress-induced working memory impairment. *Stress.* 2009; 12(5):457–63. [PubMed: 19005873]
90. Jacobs E, D'Esposito M. Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. *J Neurosci.* 2011; 31(14):5286–93. 2011. [PubMed: 21471363]
91. Inagaki T, Gautreaux C, Luine V. Acute estrogen treatment facilitates recognition memory consolidation and alters monoamine levels in memory-related brain areas. *Horm Behav.* 2010; 58(3):415–26. [PubMed: 20553724]
92. Kugaya A, Epperson CN, Zoghbi S, van Dyck CH, Hou Y, Fujita M, Staley JK, Garg PK, Seibyl JP, Innis RB. Increase in Prefrontal Cortex Serotonin2A Receptors Following Estrogen Treatment in Postmenopausal Women. *Am J Psychiatry.* 2003; 160(8):1522–4. [PubMed: 12900319]
93. Epperson CN, Amin Z, Ruparel K, Gur R, Loughhead J. Interactive effects of estrogen and serotonin on brain activation during working memory and affective processing in menopausal women. *Psychoneuroendocrinology.* 2012; 37(3):372–82. [PubMed: 21820247]
94. Dalley JW, Theobald DE, Eagle DM, Passetti F, Robbins TW. Deficits in impulse control associated with tonically-elevated serotonergic function in rat prefrontal cortex. *Neuropsychopharmacology.* 2002; 26(6):716–28. [PubMed: 12007742]
95. Duff SJ, Hampson E. A beneficial effect of estrogen on working memory in postmenopausal women taking hormone replacement therapy. *Horm Behav.* 2000; 38(4):262–76. [PubMed: 11104644]
96. Keenan PA, Ezzat WH, Ginsburg K, Moore GJ. Prefrontal cortex as the site of estrogen's effect on cognition. *Psychoneuroendocrinology.* 2001; 26(6):577–90. [PubMed: 11403979]
97. Lacreuse A, Chhabra RK, Hall MJ, Herndon JG. Executive function is less sensitive to estradiol than spatial memory: performance on an analog of the card sorting test in ovariectomized aged rhesus monkeys. *Behav Processes.* 2004; 67(2):313–9. [PubMed: 15499681]
98. Baxter MG, Roberts MT, Gee NA, Lasley BL, Morrison JH, Rapp PR. Multiple clinically relevant hormone therapy regimens fail to improve cognitive function in aged ovariectomized rhesus monkeys. *Neurobiol Aging.* 2013; 34(7):1882–90. [PubMed: 23369546]
99. Dixon AF. Proceptive displays of the female common marmoset (*Callithrix jacchus*): effects of ovariectomy and oestradiol 17 beta. *Physiol Behav.* 1986; 36(5):971–3. [PubMed: 3714875]
100. Kendrick KM, Dixon AF. Effects of oestradiol 17B, progesterone and testosterone upon proceptivity and receptivity in ovariectomized common marmosets (*Callithrix jacchus*). *Physiol Behav.* 1985; 34(1):123–8. [PubMed: 4034688]
101. Dixon AF, Lloyd SA. Effects of male partners upon proceptivity in ovariectomized estradiol-treated marmosets (*Callithrix jacchus*). *Horm Behav.* 1989; 23(2):211–20. [PubMed: 2501211]

Fig 1.
Design of the study.

Fig 2.

Mean plasma oestrogens (pg/ml) in 6 control and 6 E₂-treated OVX monkeys. One outlier control monkey was removed from the analysis.

Fig 3.
Mean daily number of total, initiated and received affiliative behaviours in control and E₂-treated OVX monkeys.

Fig 4.

Top: Number of trials to criterion (left) and number of errors to criterion (right) for each discrimination of the Object Reversals in control and E₂-treated OVX monkeys. Bottom: Number of perseverative errors in each reversal in control and E₂-treated OVX monkeys. * p<.05

Fig 5. Percentage of correct responses in the Delayed Response in control and E₂-treated OVX monkeys.

Fig 6. Number of reaching errors (top) and latencies in seconds (bottom) to reach the reward as a function of sessions and treatment group.

Fig 7.

Top: Number of trials and errors to reach a 90% correct responses learning criterion in the DMP in control and E₂-treated OVX monkeys. Bottom: proportion of correct trials as a function of spatial pattern separation for the first 12 trials, the median 12 trials and the last 12 trials in control and E₂-treated OVX monkeys. Two OVX monkeys who did not reach learning criterion are not represented.

Table 1**Behavioral ethogram**

Behavior	Definition
Vocalization	Any sound made from mouth, including chirps, whistles, and chattering
Aggress	Grapple with another marmoset, involving biting, clawing, and wrestling, and chasing
Displace	Takeover of position of another animal
Locomotion	More than one step in a directed plane
Visual Explore	Sitting passively for more than 3 seconds, not scored in conjunction with any other behavior
Headcock	Turning of the head in inspection of an observer, animal, or object
Genital display	Raise tail to expose genitals
Scentmark	Rub or drag anogenital, suprapubic, or sternal region along substrate, object or partner
Scratch	Vigorous rubbing of a body part
Tuft-Flick	Rapid back-and-forth movement of ear tufts
Tactile Oral	Sniff, bite, chew, handle, or otherwise manipulate inanimate object, excluding food items and water bottle, for at least 1 sec
Eat	Consumption of food
Drink	Licking or sucking on water bottle
Social Contact	Passive close contact with another marmoset, within an arm's length, with both animals remaining stationary and in passive contact for at least 3 sec
Sniff/Nuzzle	Orient face against or toward partner, excluding anogenital region
Mount	Climb on partner's back from behind and grip partner around waist and legs; may be accompanied by pelvic thrusting
Social Play	Social interactions involving non-aggressive physical contact with other individuals; high activity
Self Play	Repetitive movements toward objects or fixtures in cage, may include spinning, swinging, and hanging
Social Groom	Use hands and/or mouth to pick through fur and/or mouth of partner, excluding anogenital region
Self Groom	Licking, picking or spreading of one's own hair or skin
Other	Sneezing, coughing, piloerection or any other behavior not identified

Table 2

Mean number of trials and standard deviation (in parentheses) required to reach a 90 % correct criterion on each delay of the Delayed Response task

	0 delay	1s delay	3s delay	6s delay	10s delay
Trials to criterion	21.45 (24.23)	50.45 (43.39)	76.18 (85.48)	44.55 (57.16)	74.91 (69.64)