Behavioral/Systems/Cognitive

Executive Function and Attention Are Preserved in Older Surgically Menopausal Monkeys Receiving Estrogen or Estrogen Plus Progesterone

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Animal models of menopause have been used to further define the cognitive processes that respond to hormone therapy and to investigate parameters that may influence the cognitive effects of estrogen. Many investigations in animals have focused on memory; however, the effects of hormone therapy on executive function and attention processes have not been well studied. Thus, the purpose of this set of investigations was to assess the effects of estrogen therapy alone or with progesterone on executive and attention processes in middle-aged ovariectomized monkeys. Monkeys were preoperatively trained on a modified version of the Wisconsin card sort task and on a visual cued reaction time task. Hormone therapy was initiated at the time of ovariectomy and cognitive function was reassessed at 2, 12, and 24 weeks postoperatively. Relative to monkeys receiving either of the estrogen therapies, monkeys receiving placebo were impaired in their ability to shift a cognitive set in the Wisconsin card sort task and were impaired in shifting visuospatial attention in the visual cued reaction time task. Our findings are consistent with clinical studies that indicate that hormone therapy can improve executive function and attention processes in postmenopausal women.

Introduction

Ovarian hormones modulate many systems and circuits in the brain that are important for cognitive function (McEwen, 2001; Woolley, 2007). Executive function and attention processes are among some of the cognitive domains that are enhanced by hormone therapy (HT) in postmenopausal women. The term executive function refers to a larger collection of high level cognitive processes that includes reasoning, problem-solving, and cognitive flexibility (i.e., cognitive set-shifting or attentional set-shifting). The Wisconsin card sorting task (WCST) (Berg, 1948; Milner, 1963) traditionally has been used to assess cognitive set-shifting in humans and performance on the task is improved in postmenopausal women on HT (Schmidt et al., 1996; Dunkin et al., 2005; Erickson et al., 2007). Attention is also a term that refers to many different processes and different types of attention have been measured in postmenopausal women, including vigilance (sustained attention) and visual search (selective attention). Both of these types of attention processes were improved by HT in some studies of postmenopausal women (Vanhulle and Demol, 1976; Fedor-Freybergh, 1977; Schmidt et al., 1996; Rudolph et al., 2000; Smith et al., 2001), but not all studies (Polo-Kantola et al., 1998; Binder et al., 2001; Alhola et al., 2006; Kurt et al., 2006).

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Animal models of menopause have been used to further define the cognitive processes that respond to HT and to investigate parameters that may influence the cognitive effects of HT. Although many animal studies have focused on memory, the effects of HT on executive function and attention processes have not been well studied. Indeed, these cognitive functions have not been examined at all in ovariectomized (OVX) rodents. In monkeys, measures of cognitive flexibility were not affected by either OVX or estrogen alone therapy (ET) in young (Voytko, 2000) or older (Lacreuse et al., 2000, 2004) monkeys. In contrast, visuospatial attention was disrupted after OVX in young monkeys and this impairment was reversed with ET (Voytko, 2002).

In a series of investigations, we have been examining the effects of ET and estrogen plus progesterone therapy (E+P) on cognitive function of middle-aged surgically menopausal monkeys. We previously reported that visual recognition memory was preserved by ET or E+P in middle-aged surgically menopausal monkeys (Voytko et al., 2008). The purpose of this current set of investigations was to assess the effects of these therapies on executive function and attention processes in middle-aged menopausal monkeys to further our understanding of how these cognitive processes respond to HT in primates. To assess executive function, a modified version of the WCST was used to measure cognitive set-shifting, and a visuospatial cued reaction time task (VCRTT) was used to measure shifting of visuospatial attention. Thus, the ability to shift between perceptual/stimulus dimensions or between spatial locations was evaluated in middle-aged menopausal monkeys. We predicted that both shifting of cognitive sets (WCST) and shifting of spatial attention (VCRTT) would be better in monkeys receiving ET and/or E+P compared with monkeys receiving placebo.

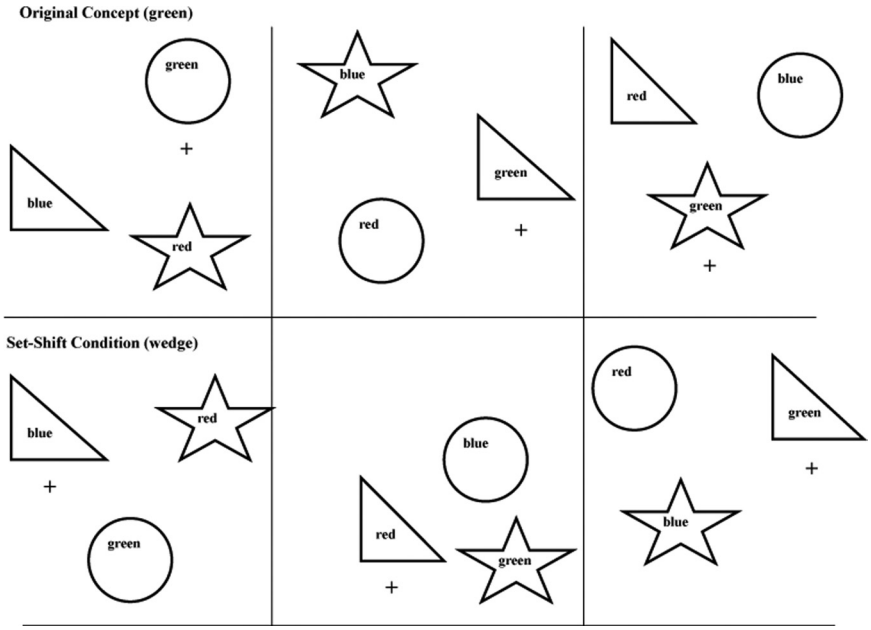

Figure 1. Schematic of the computerized WCST. Each block illustrates an individual trial of the WCST. Three stimuli that vary in 1–3 shapes and 1–3 colors are shown on each trial. The top three blocks illustrate trials in which the monkey learns an original concept (in this case the color green is relevant to receive a reward, +) and the bottom three blocks illustrate trials in which the monkey learns a conceptual set-shift (the wedge shape is now relevant to receive a reward).

Table 1. Stimuli used in each assessment phase of WCST

Test phase	Colors	Shapes
Preoperative	Burgundy (+ for shift)	Horseshoe (+ for original concept)
	Light blue	Lighting bolt
	Mint green	Circle with slash in the center
Postop #1	Bright red (+ for original concept)	Cylinder (+ for shift)
	Amber	Airplane
	Lavender	Seven-point star
Postop #2	Bright yellow (+ for shift)	Pac-Man (+ for original concept)
	White	Right arrowhead
	Deep purple	File symbol
Postop #3	Pale pink (+ for shift)	Folded banner (+ for original concept)
	Deep fuschia	Pipe
	Steel Blue	Asterisk

+, Correct examplar.

Materials and Methods

Subjects

Twenty-four female rhesus (*Macaca mulatta*) monkeys (mean ± SEM at time of OVX and treatment initiation: 19.7 ± 0.5 years) were the subjects of these experiments. These monkeys also had been tested on a delayed matching-to-sample task and on a delayed response task as part of a larger study investigating the cognitive effects of HT in middle-aged female monkeys (Voytko et al., 2008). The animals were individually housed in a climate-controlled room. Water was available *ad libitum* and rations of monkey chow were provided after the daily behavioral sessions. They received environmental enrichment in the form of novel toys and foods regularly. All procedures involving animals were conducted according to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) and approved by the Wake Forest University School of Medicine Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering.

Experimental design

Monkeys were trained and tested on the tests of executive function and attention before ovariectomy and initiation of HT, and were reexamined at 2, 12, and 24 weeks after surgery. Animals were tested 5 d per week. Postoperative (postop) testing of monkeys receiving ET or E+P was conducted throughout their treatment schedule and was not confined to only a particular phase of their schedule to mirror the conditions that hormonally treated postmenopausal women normally experience in their daily lives. Monkeys were randomized to treatment group based on their preoperative (preop) mean performance on the delayed response and delayed matching-to-sample tasks (Voytko et al., 2008).

Ovariectomy

A staff veterinarian ovariectomized the monkeys using sterile surgical procedures. The monkeys were sedated with ketamine (5–10 mg/kg, i.m.), the trachea intubated, and the animals maintained on 2.5% isoflurane and oxygen during the surgery. A midline incision was made, the ovaries were exteriorized, the vasculature was ligated, the ovaries removed, and the incision was then sutured closed. The monkeys were monitored postoperatively until recovered from anesthesia and then on a daily basis until sutures were removed. Butorphanol (0.025 mg/kg, i.m.) was administered once to alleviate pain at 3–4 h after surgery. Surgically related postoperative complications were not noted in any of the monkeys.

Hormone treatments

At the time of ovariectomy, monkeys were subcutaneously implanted with a 3.5 cm length of either empty SILASTIC tubing (0.335 cm inner diameter and 0.465 cm outer diameter) [placebo group (PL); $n = 7$] or tubing containing a 3 cm packed column of 17β-estradiol ($n = 17$; Steraloids). Implants of this size have been used successfully in previous monkey studies and have remained viable for >14 months after implantation (Voytko, 2000, 2002; Voytko et al., 2008). These implants delivered and maintained mean basal estradiol (E2) levels of 63.5 ± 3.6 pg/ml (Voytko et al., 2008). All monkeys with E2 implants also received an injection of estradiol valerate (0.10–0.20 mg/cc, Pfizer,) on day 12 to simulate the preovulatory peak in E2 that normally occurs in the menstrual cycle. These injections achieved mean E2 peaks of 536.31 ± 24.6 pg/ml in these animals (Voytko et al., 2008). Nine of the monkeys receiving E2 implants comprised the ET group while the remaining eight monkeys with implants comprised the E+P group and were given oral doses of progesterone (0.2 mg/kg, Prometrium, USP, Solvay Phamaceuticals) for 12 d beginning on day 16. Oral dosing of progesterone (P4) was chosen because of the reoccurring cyclical nature of the therapy schedule of this hormone. The P4 dosing schedule duration and start day was based on the normal menstrual cycle of macaque monkeys (Goodman et al., 1977; Downs and Urbanski, 2006). The P4 was placed in various foods or treats for dosing and each animal was closely observed during the dosing to ensure that the foods/treats were totally consumed. This schedule of E2 injections and P4 dosing was repeated monthly through the study.

Hormone assays

Levels of E2 and P4 were monitored throughout the study. Awake monkeys were placed in a specially constructed transfer cage where blood was drawn from the saphenous vein. Monkeys receiving E2 had blood samples taken the day before the E2 injection to determine E2 levels delivered by the implants. After the E2 injection, blood was again drawn 6 h later to determine peak levels of E2 attained by the injection. For E+P monkeys, blood was also drawn on days 1, 6, and 12 of P4 dosing to determine levels of P4, with another blood sample drawn 24 h after the final P4 dose to verify that P4 levels were no longer elevated. This schedule of blood sampling was repeated monthly through the course of the study. Blood was collected in sterile serum tubes, allowed to clot, centrifuged, and then serum was aliquoted into separate tubes and frozen at –20°C. Serum

Table 2. Group effect sizes

	Dependent measure	Cohen's *d*
Postop 1		
WCST learn original concept	Trials	0.09
	Errors	0.21
WCST conceptual set-shift	Trials	0.74*
	Errors	0.62
	Perseverative errors	0.76*
	Stage I blocks	0.44
	Stage II blocks	0.95*
	Stage III blocks	0.94*
VCRTT	Validity	0.31
	Benefits	0.84*
	Costs	1.08*
Postop 2		
WCST learn original concept	Trials	0.35
	Errors	0.21
WCST conceptual set-shift	Trials	0.56
	Errors	0.52
	Perseverative errors	0.54
	Stage I blocks	0.55
	Stage II blocks	0.84*
	Stage III blocks	0.11
VCRTT	Validity	0.54
	Benefits	0.59
	Costs	0.12
Postop 3		
WCST learn original concept	Trials	0.10
	Errors	0.35
WCST conceptual set-shift	Trials	0.20
	Errors	0.29
	Perseverative errors	0.22
	Stage I blocks	0.61
	Stage II blocks	0.10
	Stage III blocks	0.24
VCRTT	Validity	0.07
	Benefits	0.22
	Costs	0.15

*Large effect size.

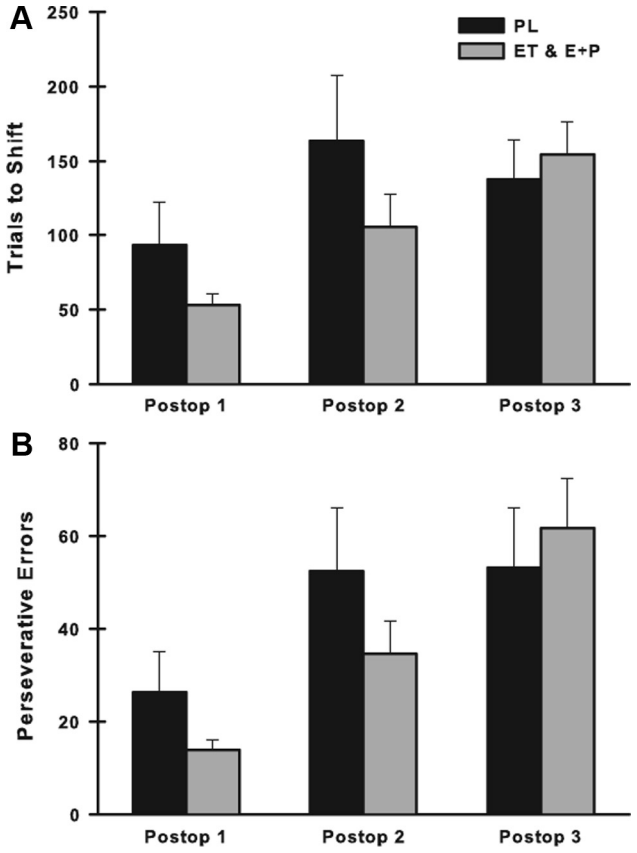

Figure 2. Conceptual set-shifting across the three postoperative evaluations. ***A***, ***B***, At the first postoperative assessment, placebo monkeys required twice as many trials to perform a conceptual shift (***A***) and made more perseverative errors in shifting (***B***) than the combined groups of estrogen therapy monkeys. Group effect sizes were only small to medium at the other evaluations. Error bars indicate SEM.

levels of E2 and P4 were measured with a Roche Diagnostics Elecsys 2010 clinical assay platform by the Oregon National Primate Research Center Endocrine Services Core Laboratory. These assays had been previously validated against traditional extraction radioimmunoassays (Hess et al., 1981). The sensitivity for E2 was 12 pg/ml and for P4 was 0.05 ng/ml. The coefficient of variation for both hormones was <10%.

Behavioral apparatus

Computer-controlled apparatuses with touchscreen monitors were used to behaviorally train and test the monkeys (Voytko et al., 2008). For all tasks, auditory feedback was provided by the sound of chimes for correct responses and a low-pitched tone for incorrect responses. Visual feedback on all tasks consisted of the monitor screen turning black after correct responses and turning blue after incorrect responses. The monitor screen turned red to signal the end of the test session.

Behavioral tasks

WCST. This task is based on one used to assess the ability to abstract, maintain, and shift a cognitive set in humans (Berg, 1948; Milner, 1963) and was modified for use in monkeys (Moore et al., 2005, 2006). Dr. James Herndon at the Yerkes National Primate Research Center (Emory University, Atlanta, GA) graciously provided touchscreen computer software for the WCST pretraining simple three choice discrimination task and the WCST itself. In these tasks, three stimuli that varied by shape and color were displayed.

Before training on the WCST task, the monkeys learned a simple three choice discrimination task to establish that the monkeys could discriminate among three stimuli that differed by color (yellow, purple, aqua) and shape (bow tie, cross, square) in preparation for learning the WCST. The stimuli appeared in a pseudo-random order on the monitor on each trial. The monkeys learned that touching the aqua box resulted in a reward being delivered. The correct stimulus remained the same from trial to trial. A 15 s intertrial interval (ITI) was used and learning criterion was ≥90% correct in a session of 100 trials. Once criterion was achieved, the animal was moved to the WCST the next testing day. Dependent measures for the three choice discrimination task were total number of trials and errors to achieve criterion.

The WCST itself consisted of two phases: (1) initial learning of a conceptual set, and (2) shifting of a conceptual set (Fig. 1). In both phases, three stimuli, differing in color and shape, appeared in a pseudo-random order in nine possible locations on the monitor on each trial. All possible combinations of stimuli were presented in a balanced randomized manner over a 4 d cycle and were repeated until criterion was achieved. During the initial conceptual set learning, the monkeys had to learn which stimulus dimension (color or shape) and which particular dimension examplar (the specific color or shape) was relevant to receive a reward. Touching the correct stimulus produced a pellet reward. Once the monkey touched the correct stimulus on 10 consecutive trials, the program changed the rewarded stimulus contingency during the same test session without the animal's knowledge (conceptual set-shift; from color to shape or vice versa). At the preoperative and postoperative assessments, the monkeys were tested for their ability to learn a new conceptual set and to perform one conceptual set-shift. At each of these assessments, colors and shapes were used that had never been used previously (Table 1). A 15 s ITI was used and each test session consisted of 100 trials. Criterion for both initial learning and shifting of a conceptual set was 10 consecutive correct responses. Dependent measures to learn the original conceptual set were the number of trials and errors to achieve

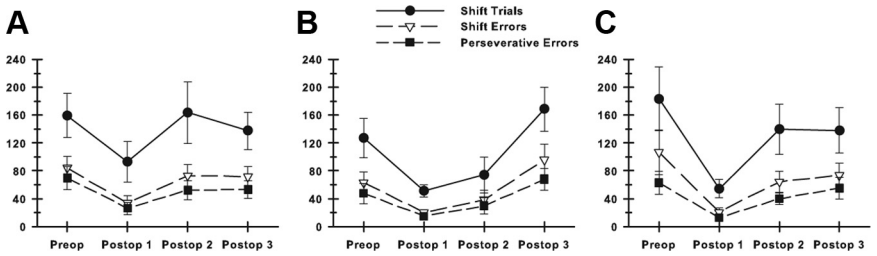

Figure 3. Conceptual set-shifting within groups across evaluations. ***A–C***, PL monkeys (***A***), ET monkeys (***B***), E+P monkeys (***C***). Differences in performance were found across evaluations for each group. See Results for details. Error bars indicate SEM.

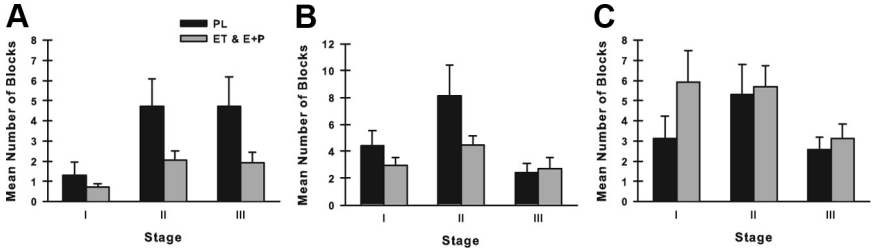

Figure 4. ***A–C***, Stage performance during conceptual set-shifts at Postop 1 (***A***), Postop 2 (***B***), and Postop 3 (***C***) assessments. Monkeys with PL spent significantly longer in stages II and III at Postop 1 and longer in stage II at Postop 2 than the combined groups of estrogen therapy monkeys. Error bars indicate SEM.

criterion. Dependent measures of shifting a conceptual set was the number of trials, number of errors, and number of perseverative errors (choosing of a stimulus containing a component of the previously rewarded category) to perform a shift.

VCRTT. The ability to orient and to shift visuospatial attention is assessed by this task (Posner, 1980). The procedure was similar to that described previously (Voytko et al., 1994; Baxter and Voytko, 1996; Voytko, 2002) and adapted for the touchscreen system. At the start of a trial, a white square appeared at the center of the monitor. The monkey was required to continuously touch the central square for 1–3 s. At the end of the delay, one of two green circles ("cue") appeared to the left or right of the central square and was present for 200 ms, during which time the monkey was required to continue touching the central square. After the 200 ms, the central square was removed and a white square ("target") appeared on the left or right of the monitor. The correct strategy to receive a reward was to release touching the center of the monitor and to touch the target. Cues were valid (on the same side as where the target will appear), invalid (on the side opposite to where the target will appear), or neutral (both cue lights were illuminated as cues). Cues were valid in ~70%, invalid in 15%, and neutral in 15% of the trials within a session. A 15 s ITI was used and each daily session consisted of 160 trials. Training on the VCRTT began with trials in which a target did not appear after the cue presentation. In these initial trials, monkeys received a reward for continually touching the central square while the cue was illuminated. Trials would be terminated and a 5 s time-out would occur if the monkey removed its hand from the center square prematurely. Valid, neutral, and invalid trials were introduced once an 85% level of accuracy was achieved in these training trials. Testing took place for 5 d for an approximate total of 560 valid trials, 120 invalid trials, and 120 neutral trials. The dependent measures were the time (in milliseconds) to release hold on the center of the monitor at target appearance (release time), validity (invalid − valid trials), benefits (neutral − valid trials), costs (invalid − neutral trials), and time (in milliseconds) to hit the target (hit time).

Statistical analyses

Data were initially analyzed with two-way measures of analyses of variance with group as a between-subjects factor and time as a repeated measures factor. To supplement the postoperative omnibus ANOVAs, a standardized measure of effect size, Cohen's *d* [(Group A mean − Group B mean)/mean SD of Groups A and B], was calculated to index the size of the observed differences between treatment groups (Cohen, 1988). The *d* statistic is commonly used in trial planning and allows for evaluation of the size of an effect in a unit-less SD metric (i.e., outcomes of different measurement units can be directly compared using *d*). Reported in units of SD ranging from 0 (no group differences) to any positive number, a $d \leq 0.20$ represents small differences, $d = 0.50$ represents medium differences, and $d > 0.80$ represents large differences (although the clinical significance of an effect depends on what is actually being measured). Additional postoperative comparisons of interest identified a priori are also presented with group effect sizes.

For the WCST, an additional analysis of conceptual set-shifting was conducted to evaluate whether particular stages of conceptual set-shifting may be influenced by the experimental treatments based on similar analyses of reversal discrimination learning (Jones and Mishkin, 1972; Lai et al., 1995; Voytko, 1999). Two-way analyses of variance were conducted to compare groups for the number of blocks of 10 trials that were concentrated in 1 of 3 learning stages. Stage I (defined as performance where 7–10 errors were made in a 10-trial block) is the stage in which the animal is trying to disengage from the initial concept and overcome strong perseverative tendencies. Stage II (defined where 4–6 errors were made in a 10-trial block) is the stage in which the animal is establishing the new stimulus-response association and is performing near chance levels. Stage III (defined where 0–3 errors were made in a 10-trial block) is the stage in which the animal is attaining acquisition criterion for the set-shift.

For each task, the ET and E+P groups were compared initially to determine whether these two HT groups differed. If these groups were equivalent in their performance, their data were combined into a single group of estrogen treated monkeys (E) and compared with the PL monkeys. If there were differences between the HT groups, then the ET and E+P groups were each included in the comparison to PL monkeys. Within-group analyses were performed for the three individual treatment groups (PL, ET, E+P) with and without inclusion of the preoperative data so that comparisons could be made of data collected before and after ovariectomy/treatment, as well as only once treatment was initiated. Differences were considered significant at $p < 0.05$. *p* values of 0.05–1.0 are listed to illustrate trends in the analyses.

Results

Hormone levels

We previously reported that serum levels of E2 and P4 achieved by our hormone regimen produced physiologic menstrual cycle levels of these hormones in these monkeys (ET: E2 implants = 69.3 + 2.9 pg/ml, E2 injections = 545.47 + 27.7 pg/ml; E+P: E2 implants = 63.5 + 2.0, E2 injections = 526.0 + 43.8 pg/ml, P4 = 5.92 + 0.7 ng/ml), that E2 levels were equivalent between the ET and E+P monkeys, and that E2 levels were significantly greater in the ET and E+P monkeys compared with the PL monkeys (E2 = 8.6 + 1.7 pg/ml, P4 = <0.05 ng/ml) (Voytko et al., 2008).

WCST

Preoperatively, groups of monkeys did not differ in their ability to learn the three choice discrimination task (trials or errors, *p* values >0.05). In the WCST, monkeys that were eventually randomized to the ET group were slightly better in their ability to learn the first conceptual set than those destined to receive E+P (but not PL) as based on number of errors to learn (group, $F_{(2,21)} =$

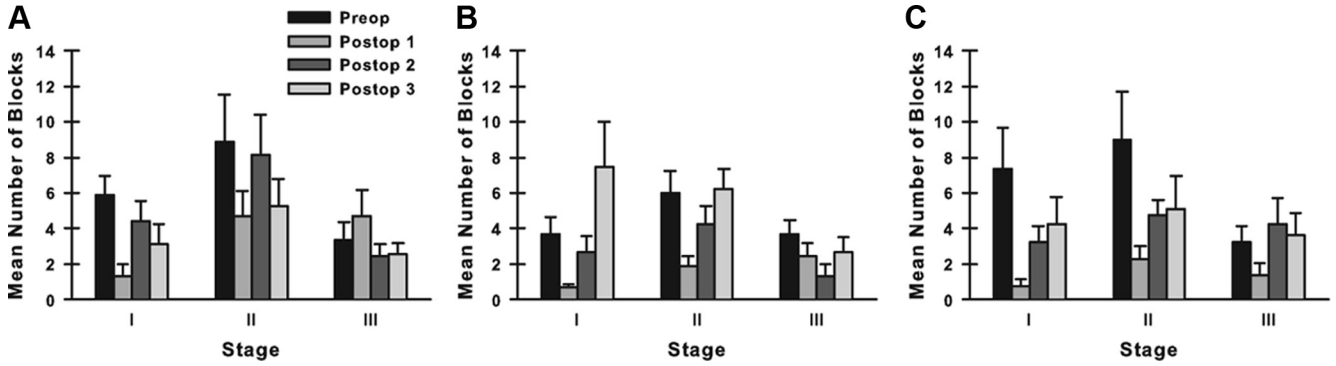

Figure 5. Within-group stage performance during conceptual set-shifts at the three postoperative assessments. ***A–C***, PL monkeys (***A***), ET monkeys (***B***), E+P monkeys (***C***). Groups of monkeys spent different periods of time in different stages. See Results for details. Error bars indicate SEM.

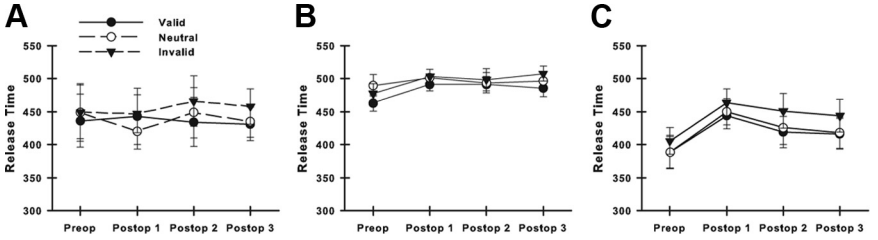

Figure 6. Release time in the VCRTT within-group across evaluations. ***A–C***, PL monkey (***A***), ET monkeys (***B***), E+P monkeys (***C***). E+P monkeys had slower release times postoperatively but PL and ET monkeys did not differ across assessments. Error bars indicate SEM.

3.74, $p = 0.04$; Tukey posthocs: ET vs E+P, $p = 0.04$; ET vs PL, $p > 0.05$) but there was no difference between the groups based on number of trials to learn (group, $F_{(2,21)} = 2.74$, $p > 0.05$). The groups also did not differ preoperatively in their ability to perform a conceptual set-shift (p values >0.05).

Postoperatively, monkeys receiving ET or E+P were equivalent in learning the original conceptual set and in performing a shift across assessments ($p > 0.05$). The scores of these two groups were combined (E group) and compared with those of the PL monkeys. The groups were comparable in their ability to learn the original concept across the postoperative evaluations as measured in either trials or errors (group, time, time × group, all p values >0.05) and this also was reflected in the small group effect sizes (Table 2). In performing conceptual shifts, the omnibus ANOVA indicated that only the effect of time ($F_{(2,44)} = 5.88$, $p < 0.01$) was significant for trials to shift (group and group × time, p values >0.05). However, monkeys with PL required almost twice as many trials than the E monkeys to set-shift at the first postoperative assessment (Fig. 2*A*) and indeed the group effect size ($d = 0.74$, $F_{(1,22)} = 3.47$, $p = 0.07$) at Postop #1 indicated that there was a considerable difference between the treatment groups in number of trials to perform the conceptual set-shift. Group effect sizes (Table 2) for trials to shift were small to medium for the other postoperative assessments. For errors to perform the conceptual shift, only the effect of time was significant ($F_{(2,44)} = 10.40$, $p < 0.01$) (group and group × time, p values >0.05) and group effect sizes (Table 2) indicated only small to medium group differences for this dependent measure at each postoperative evaluation. For perseverative errors to shift, only the effect of time ($F_{(2,44)} = 10.74$, $p < 0.01$) was significant (group and group × time, p values >0.05). Here also, the group effect size ($d = 0.76$, $F_{(1,22)} = 3.59$, $p = 0.07$) at Postop #1 was large indicating that monkeys with PL made more perseverative errors when performing the set-shift than the E monkeys (Fig. 2*B*).

Within-group analyses revealed that trials and errors to learn the initial concept were equivalent across assessments for each group (p values >0.05 for preop through postop or postop only). For the most part, similar patterns were seen for the three groups of monkeys in shifting conceptual sets across the three postoperative assessments. Monkeys with PL (Fig. 3*A*) differed across time for errors to shift (preop through postop: $F_{(3,18)} = 5.75$, $p < 0.01$; postop: $F_{(2,12)} = 5.72$, $p = 0.01$) and for perseverative errors to shift (preop through postop: $F_{(3,18)} = 4.77$, $p = 0.01$; postop: $F_{(2,12)} = 3.62$, $p = 0.05$) but not for trials to shift (preop through postop or postop only, p values >0.05). Monkeys with ET (Fig. 3*B*) differed across time for trials to shift (preop through postop: $F_{(3,24)} = 7.25$, $p < 0.01$; postop: $F_{(2,16)} = 11.25$, $p < 0.01$), errors to shift (preop through postop: $F_{(3,24)} = 8.43$, $p < 0.01$; postop: $F_{(2,16)} = 11.12$, $p < 0.01$), and perseverative errors to shift (preop through postop: $F_{(3,24)} = 6.94$, $p < 0.01$; postop: $F_{(2,16)} = 10.17$, $p < 0.01$). Monkeys with E+P (Fig. 3*C*) differed across time for errors to shift (preop through postop: $F_{(3,21)} = 2.88$, $p = 0.06$; postop: $F_{(2,14)} = 3.52$, $p = 0.05$) and perseverative errors to shift (preop through postop: $F_{(3,21)} = 2.83$, $p = 0.06$; postop: $F_{(2,14)} = 4.33$, $p = 0.03$), but not for trials to shift (preop through postop or postop only, p values >0.05).

Conceptual set-shift stage performance was equivalent between ET and E+P monkeys across postoperative assessments (p values >0.05) and their combined scores were compared with those of the PL monkeys. The overall main effects of time ($F_{(2,44)} = 3.80$, $p = 0.03$) and stage ($F_{(2,44)} = 9.84$, $p < 0.01$) were significant, but not group ($p > 0.05$). The interactions of time × group ($F_{(2,44)} = 3.23$, $p = 0.04$) and time × stage ($F_{(4,88)} = 4.07$, $p < 0.01$) also were significant but other interactions were not. Subsequent analyses were conducted to further probe the time × group interaction. At Postop #1 (Fig. 4*A*), significant effects of group ($F_{(1,22)} = 6.99$, $p = 0.01$) and stage ($F_{(2,44)} = 11.75$, $p < 0.01$), and a borderline significant interaction of stage × group ($F_{(2,44)} = 2.41$, $p = 0.10$) were found. Large group effect sizes were found for stage II ($d = 0.95$, $F_{(1,22)} = 5.56$, $p = 0.02$) and stage III ($d = 0.94$, $F_{(1,22)} = 5.30$, $p = 0.03$) indicating that monkeys receiving PL required more blocks than E monkeys at these stages. In Postop #2 (Fig. 4*B*), significant effects of stage ($F_{(2,44)} = 12.25$, $p < 0.01$) and the interaction of stage × group ($F_{(2,44)} =$

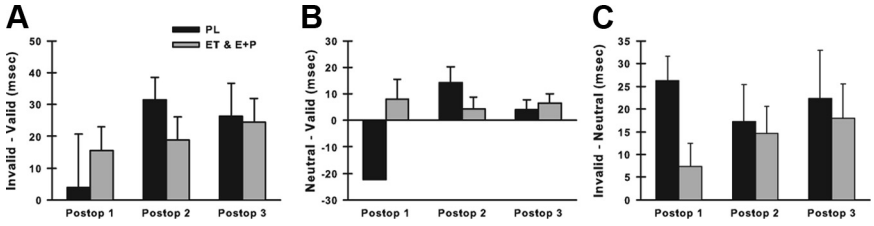

Figure 7. ***A–C***, Measures of validity (***A***), benefits (***B***), and costs (***C***) in PL-and estrogen-treated monkeys across the three postoperative assessments. Monkeys with PL had fewer benefits and greater costs than the combined groups of estrogen monkeys at the first postoperative assessment. Error bars indicate SEM.

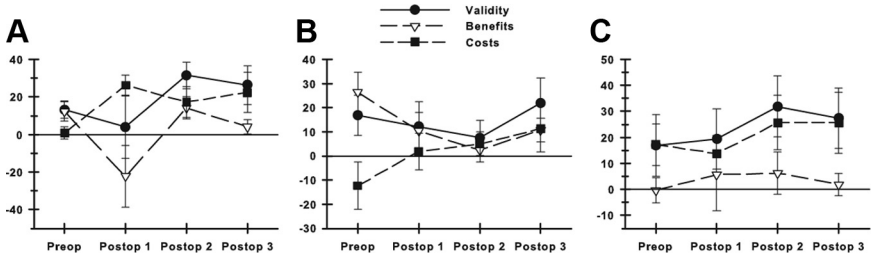

Figure 8. ***A–C***, Within-group measures of validity, benefits, and costs in PL monkeys (***A***), ET monkeys (***B***), and E+P monkeys (***C***) across assessments. Monkeys with PL significantly differed across evaluations in their benefits and costs. Monkeys with ET or E+P did not vary significantly in measures across assessments. Error bars indicate SEM.

3.25, $p = 0.04$) were found. A large group effect size was found for stage II ($d = 0.84$, $F_{(1,22)} = 4.40$, $p = 0.04$) again indicating that monkeys with PL spent longer in this stage than E monkeys. There were no significant effects of group, stage, or stage × group for Postop #3 (Fig. 4*C*) (all p values >0.05) and group effect sizes for the three stages were only small to medium (Table 2) at this assessment.

Within-group analyses of stages of set-shifting indicated monkeys spent different periods of time at the different stages and in some cases, this varied across assessments. For monkeys with PL (Fig. 5*A*), a significant stage effect (preop through postop: $F_{(2,12)} = 8.24$, $p < 0.01$; postop: $F_{(2,12)} = 6.02$, $p = 0.01$) reflected the greater time spent in stage II and a time × stage interaction (preop through postop: $F_{(6,36)} = 2.60$, $p = 0.03$; postop: $F_{(4,24)} = 3.09$, $p = 0.03$) reflected the change over time spent especially in stages I and II. For monkeys with ET (Fig. 5*B*), a significant stage effect (preop through postop: $F_{(2,16)} = 4.13$, $p = 0.03$; postop: $F_{(2,16)} = 3.04$ $p = 0.05$) reflected the greater time in stages I and II and a significant time effect (preop through postop: $F_{(3,24)} = 11.35$, $p < 0.01$; postop: $F_{(2,16)} = 11.06$, $p < 0.01$) reflected the variable performance over assessments. A time × stage interaction (preop through postop: $F_{(6,48)} = 2.14$, $p = 0.06$; postop: $F_{(4,32)} = 2.63$, $p = 0.05$) reflected the variable performance of monkeys with ET over time particularly in stages I and II. Stage analyses for E+P monkeys (Fig. 5*C*) revealed differences across time (preop through postop: $F_{(3,21)} = 2.77$, $p = 0.06$; postop: $F_{(2,14)} = 2.78$, $p = 0.09$) and a time × stage interaction (preop through postop: $F_{(6,42)} = 2.92$, $p = 0.05$) indicated that time spent in stages I and II in particular decreased postoperatively.

VCRTT

Preoperatively, two monkeys were not able to learn the VCRTT and thus were not tested further on this task. Thus, 7 PL monkeys, 8 ET monkeys, and 7 E+P monkeys completed all evaluations of the VCRTT preoperatively and postoperatively. The three groups were comparable in the VCRTT preoperatively (p values >0.05). Comparisons of release time of the ET and E+P monkeys indicated that there was a difference between these groups postoperatively (group, $F_{(1,13)} = 7.03$, $p = 0.02$), and thus, these estrogen groups were not combined to compare with PL monkeys for this dependent measure. In comparing the release time of the three treatment groups, only the main effect of trialtype was significant ($F_{(2,38)} = 15.8$, $p < 0.01$). None of the other main effects or interactions were significant, including that of group (all p values >0.05; data not shown). Time analyses for each group of monkeys indicated that the E+P monkeys were slower postoperatively compared with their preoperative performance (Fig. 6*C*) (preop through postop: time, $F_{(3,18)} = 3.12$, $p = 0.05$), but they did not differ across their postoperative assessments (postop: time, $p > 0.05$). Neither PL or ET monkeys differed across assessments in their release time (Fig. 6*A*,*B*) (preop through postop or postop only, time, p values >0.05 for both groups). Release time of all groups of monkeys differed across the types of trials (PL: preop through postop, $F_{(2,12)} = 6.81$, $p = 0.01$ and postop, $F_{(2,12)} = 6.59$, $p = 0.01$; ET: preop through postop, $F_{(2,14)} = 3.72$, $p = 0.05$ and postop, $F_{(2,14)} = 3.27$, $p = 0.06$; E+P: preop through postop, $F_{(2,12)} = 5.53$, $p = 0.02$ and postop, $F_{(2,12)} = 6.52$, $p = 0.01$). The three groups of monkeys did not differ in hit time across the postoperative assessments (all p values >0.05) nor did hit time vary across time within each group of monkeys (all groups, $p > 0.05$; data not shown).

Comparisons between the ET and E+P groups indicated that the groups were comparable for validity ($p > 0.05$), benefits ($p > 0.05$), and costs ($p > 0.05$) and thus the two estrogen groups were combined into a collective E group of monkeys and compared with the PL monkeys. For measures of validity (Fig. 7*A*), the main effect of time was significant ($F_{(2,40)} = 3.37$, $p = 0.04$), but group or the interaction of time × group was not significant (all p values >0.05) and group effect sizes were only small to medium (Table 2). For measures of benefits (Fig. 7*B*), the main effect of time was borderline significant ($F_{(2,40)} = 2.4$, $p = 0.10$) and the interaction of time × group was significant ($F_{(2,40)} = 3.56$, $p = 0.03$). Subsequent analyses indicated that at the Postop #1 assessment, PL monkeys demonstrated fewer benefits ($d = 0.84$, $F_{(1,20)} = 3.87$, $p = 0.06$) than E monkeys. There were no significant differences in benefits between the treatment groups at Postop #2 or Postop #3 (p values >0.05) and the group effect sizes at these assessments were small to medium (Table 2). For measures of costs (Fig. 7*C*), the overall ANOVA did not indicate significant differences of group, time or time × group (all p values >0.05), however the substantial group effect size at Postop #1 ($d = 1.08$, $F_{(1,20)} = 4.82$, $p = 0.04$) reflected that the PL monkeys had five times greater costs than the E monkeys at this assessment. The group effect sizes for measures of costs were small at the Postop#2 and #3 assessments (Table 2).

Within-group comparisons revealed that measures of validity did not differ significantly across assessments for PL monkeys (Fig. 8*A*) (preop through postop or postop only, p values >0.05); however, PL monkeys differed across time in their benefits (Fig. 8*A*) (preop through postop: $F_{(3,18)} = 3.02$, $p = 0.05$; postop: $F_{(2,12)} = 2.89$, $p = 0.09$), reflecting a large decrease in benefits at

their first postoperative assessment, and differed across time in their costs (Fig. 8*A*) (preop through postop: $F_{(3,18)} = 2.59$, $p = 0.08$; postop: $p > 0.05$), reflecting an increase in costs postoperatively. Measures of validity, benefits, or costs did not vary significantly across time for the ET monkeys (Fig. 8*B*) (preop through postop or postop only, p values >0.05) or for the E+P monkeys (Fig. 8*C*) (preop through postop or postop only, p values >0.05).

Discussion

The results of the present study indicate that shifting of cognitive set or visuospatial attention is impaired in middle-aged surgically menopausal monkeys that do not receive estrogen therapies compared with monkeys that receive therapy, and that these cognitive functions are equivalent between monkeys receiving estrogen alone or estrogen plus progesterone.

Compared with HT groups of monkeys, the PL monkeys were impaired in shifting their cognitive strategy in the WCST, measured by their conceptual set-shifting performance. Although PL monkeys did not have impairments in learning a new original conceptual set, the stage analyses revealed that the set-shifting impairments are related to difficulty learning the new conceptual set within the context of shifting sets; indicated by the greater amount of time PL monkeys spent in stages II and III compared with HT monkeys (Jones and Mishkin, 1972). Failure in cognitive set-shifting, as measured by the WCST or by the intradimensional/extradimensional task paradigm (Roberts et al., 1988), can be related to either the inability to release attention from a relevant perceptual dimension (perseveration, approximately to stage I) or from an inability to refocus attention to a previously irrelevant perceptual dimension that now has become relevant (approximately stages II and III) (Owen et al., 1993). Thus, the impairment in shifting cognitive set by the PL monkeys in the present study may be related to a difficulty in learning about previously irrelevant stimuli (Mackintosh, 1983). In contrast to the observations made in the present study, previous investigations reported that cognitive flexibility was unaffected by OVX or ET in older menopausal monkeys when measured either by the WCST or discrimination reversal tasks (Lacreuse et al., 2000, 2004), or in young adult OVX monkeys using discrimination reversal tasks (Voytko, 2000). One factor that could explain the difference between our findings and the previous studies in older menopausal monkeys is the significant difference in length of time from OVX to initiation of cognitive testing between the studies (2 weeks in present study vs mean of ~15 years in Lacreuse et al. studies); evidence is accumulating to suggest that there may be a critical menopausal period in which to observe effects (Maki, 2006; Genazzani et al., 2007; Sherwin and Henry, 2008). Moreover, we conducted a more detailed analysis of stages of conceptual set-shifting that permitted us to identify the component processes responsible for the impaired performance in the conceptual shifts.

Monkeys receiving PL demonstrated lowered benefits and higher costs postoperatively in the VCRRT compared with HT monkeys or to their preoperative measures. These results were related to the slower release times on valid and invalid trials compared with the neutral trials and indicate inefficient processing of the advanced cues in the task by PL monkeys. Our laboratory is the only one to have examined visuospatial attention in menopausal monkeys, and our observations here are similar to those of our study in young adult surgically menopausal monkeys where we found that PL monkeys also demonstrated significantly increased costs in the VCRRT, while ET monkeys had decreased costs (Voytko, 2002). Collectively, our observations in menopausal monkeys suggest that loss of estrogen disrupts component processes of visuospatial attention and that these effects are broader with advancing age.

Consistent with the observations made in the present study of menopausal monkeys, postmenopausal women receiving HT demonstrate better performance on the WCST than women not receiving therapy (Schmidt et al., 1996; Berman et al., 1997; Dunkin et al., 2005; Ghidoni et al., 2006; Erickson et al., 2007; Wegesin and Stern, 2007) (but see Pefanco et al., 2007). Although various measures of visuospatial (e.g., clock drawing, block design) or attention (e.g., visual search, vigilance) function have been assessed in postmenopausal women (for review, see Haskell et al., 1997; LeBlanc et al., 2001; Sherwin, 2002; Zec and Trivedi, 2002; Lethaby et al., 2008), the specific ability to shift visuospatial attention has not been measured. On the basis that our PL monkeys were impaired in attentional shifting, either among stimulus dimensions (WCST) or spatial dimensions (VCRTT), suggests that other measures of attentional shifting also may be altered in untreated postmenopausal women.

The prefrontal and parietal cortices form part of a frontoparietal attention network (Mesulam, 1981; Corbetta et al., 1993; Nobre et al., 1997, 2000; Hopf and Mangun, 2000; Hopfinger et al., 2001), and both of these cortical regions are involved in cognitive set-shifting (Owen et al., 1991; Dias et al., 1996; Birrell and Brown, 2000; Rogers et al., 2000; Fox et al., 2003) and shifting of visuospatial attention (Alivisatos and Milner, 1989; Petersen et al., 1989; Koski et al., 1998; Thiel et al., 2004; Vossel et al., 2006). Executive function and attention processes are altered with advancing age (Albert et al., 1990; Greenwood and Parasuraman, 1994, 2004; Parasuraman and Greenwood, 1998; Ridderinkhof et al., 2002; Fisk and Sharp, 2004) and performance on the WCST, in particular, is impaired beginning in middle-age in both humans (Gunning-Dixon and Raz, 2003) and monkeys (Moore et al., 2006). The structure and function of the prefrontal cortex is particularly sensitive to age-related changes in humans (Gur et al., 1987; Tisserand et al., 2002; Gunning-Dixon and Raz, 2003; Tumeh et al., 2007; Chee et al., 2009) and monkeys (Peters et al., 1994; Luebke et al., 2004; Cruz et al., 2009; Kabaso et al., 2009). In addition, the monkey prefrontal cortex, more than the parietal cortex, is especially sensitive to manipulation of ovarian hormones (Kritzer and Kohama, 1999; Gibbs et al., 2002; Tinkler et al., 2004; Wang et al., 2004), and this prefrontal sensitivity is observed in middle-aged and older monkeys (Hao et al., 2003, 2006; Kompoliti et al., 2004; Browne et al., 2009). Thus, the executive function and attentional changes that we noted in the middle-aged menopausal monkeys of the current study may be related to hormonal effects in the prefrontal region.

We recently reported that visual recognition memory in a delayed matching-to-sample task was impaired in middle-aged surgically menopausal monkeys receiving PL, but not HT (Voytko et al., 2008). The monkeys of that memory study are the same monkeys that were the subjects of this present set of investigations. Interestingly, the timeframe in which the impairment in visual memory in PL monkeys was observed in our initial studies, matches the timeframe in which PL monkeys displayed impairments of shifting both cognitive sets and spatial attention; i.e., deficits were evident within 3 months after OVX but no longer detectable by 6 months after surgery. In concert, our findings in these older menopausal monkeys indicate that aspects of memory, executive function, and attentional abilities are responsive to ovarian hormone manipulations, that this responsiveness occurs within a short time after removal of the ovaries, and that it is not long-lasting. Our observations are consistent with reports

of beneficial effects of HT in these cognitive domains in postmenopausal women (Resnick and Maki, 2001; Smith et al., 2001; Erickson et al., 2007) and with the hypothesis that there may be a critical window in which to observe the benefits of HT (Maki, 2006; Genazzani et al., 2007; Sherwin and Henry, 2008). Because of similarities in reproductive, endocrine, and menopausal profiles to that of women, female monkeys are ideal animal models in which to investigate the effects of HT on cognitive function. Thus, continued studies in monkeys will be critical to further defining the cognitive changes that occur in menopause and identifying the most appropriate hormone therapies and regimens for maintaining and improving cognitive function of postmenopausal women.

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