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Relationship between Estradiol and Progesterone Concentrations and Cognitive Performance in Normally Cycling Female Cynomolgus Monkeys

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Abstract

Preclinical studies have demonstrated that cognitive function may be influenced by estradiol (E2) and progesterone (P4) concentrations, although few cognition studies involve normally cycling females. The present study examined cognitive performance in normally cycling female cynomolgus macaques (n=14), a species with similarities to humans in brain organization and a nearly identical menstrual cycle to women. Initial assessments compared cognitive measures to circulating concentrations of E2 and P4 (n=12). Once a relationship was characterized between hormones and cognitive performance, the menstrual cycle was divided into 4 distinct phases: early follicular (EF), late follicular (LF), early luteal (EL) and late luteal (LL), verified by the onset of menses and serum concentrations of E2 and P4. Concentrations of E2 were highest during the LF phase and P4 concentrations peaked during the EL phase. All monkeys were trained on two cognitive tasks: reversal learning, involving simple discrimination (SD) and reversal (SDR), which measured associative learning and behavioral flexibility, respectively (n=3–4 per phase) and a delayed match-to-sample (DMS) task which assessed working memory (n=11). P4 concentrations were positively correlated with number of trials and errors during SD performance, but not during acquisition of the SDR task or maintenance of the reversal-learning task. Across the menstrual cycle, significantly fewer errors were made in the SDR task during the LF phase, when E2 concentrations were high and P4 concentrations low. Working memory, assessed with the DMS task, was not consistently altered based on previously characterized menstrual cycle phases. These findings demonstrate a relationship between P4, E2 and cognitive performance in normally cycling cynomolgus monkeys that is task dependent. Knowledge of these interactions may lead to a better understanding of sex-specific cognitive performance.

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Keywords

Estradiol; Progesterone; Cognition; CANTAB; Working memory; Behavioral flexibility; Female cynomolgus monkey

INTRODUCTION

Executive function is largely responsible for the flexible adaptation to changes in the environment and encompasses a number of distinct tasks that involve the prefrontal cortex (PFC), frontostriatal networks and hippocampus. Executive function can be objectively measured by studying tasks that assess reinforcement learning in which behavior is shaped by stimulus-outcome associations (Eisenegger et al., 2014). Executive function includes 1) monitoring and adapting to cues relevant to a current goal and discarding/suppressing irrelevant information, 2) shifting, the ability to redirect focus between multiple modalities or tasks, and 3) inhibition, the ability to suppress or withhold a preplanned or impulsive response (see Miyake et al., 2000; Gould and Nader, 2015).

It has been known for some time that sex differences exist in cognitive performance, with women performing better on verbal tasks while men have better visuospatial skills (cf. Baros et al., 2015). When studying females, these sex differences may be attributed to fluctuations in estradiol (E2) and progesterone (P4). Hampson (1990) tested the hypothesis that at certain points in the menstrual cycle, hormone fluctuations in women would facilitate performance over males by studying performance on a series of cognitive tests in normally cycling women. In that study, women were tested twice on a battery of six cognitive and motor measures; testing occurred approximately 6 weeks apart, once to coincide with menses and the other during the preovulatory elevation of E2. Performance on spatial ability tasks was better during menses, when E2 and P4 concentrations are low, than during the preovulatory phase, while women performed better on motor tasks during the preovulatory phase compared to menses. In fact, they reported a curvilinear relationship between E2 concentrations and cognition. Although they did not measure P4 concentrations, this study highlights the task-dependent nature of E2 (and perhaps P4) effects on cognition (see Lacreuse et al., 2015). In a recent review on the role of P4 in cognition, Baros et al. (2015) divide the review into studies that show detrimental effects and those that show positive effects. One of the limitations noted in that review was the lack of preclinical studies in normally cycling animals.

Female subjects are typically under-utilized in neuroscience research, partly due to changes in neurochemistry, neurohormones and behavior across the menstrual cycle. As mentioned above, there is evidence for differences in cognitive performance across the menstrual cycle (Drake et al., 2000; Lacreuse et al., 2001; Maki et al., 2002; Rosenberg and Park, 2002). In normally cycling women, the mechanism mediating interactions between menstrual cycle phase and cognition has been associated with E2 and P4 concentrations in specific brain regions (e.g., McEwen and Alves, 1999; Osterlund et al., 2000; Milad et al., 2010; Zhang et al., 2010; He et al., 2011; see Toffoletto et al., 2014 for review), stress pathways (e.g., Felmingham et al., 2012) and neurotransmitters. For example, clinical observations suggest

that E2 fluctuations interact with dopamine (DA) to exert powerful effects on mood, mental state, behavior and memory (Fink et al., 1996; Carroll and Anker, 2010; Van Voorhees et al., 2012; Manovani and Fucic, 2014). Consistent with these findings, PET studies in female monkeys have shown significantly higher brain DA D2/D3 receptor availability in the luteal phase compared to the follicular phase of the menstrual cycle (Czoty et al., 2009). Less is known about the mechanism by which P4 may interact with the DA system or cognitive performance (van Wingen et al., 2008), but allopregnanolone, an active metabolite of progesterone, has been shown to influence GABA neurotransmission (see Baros et al., 2015).

The present study examined the effects of fluctuations in E2 and P4 concentration on cognitive performance in 14 normally cycling female cynomolgus monkeys. Old World monkeys share many characteristics with humans in terms of endocrine physiology, cognition, neuroanatomy and a complex social hierarchy (Lacreuse and Herndon, 2002; Phillips et al., 2014; Lacreuse et al., 2015) and they have an approximate 28-day menstrual cycle with similar fluctuations of E2 and P4 as observed in women (Appt, 2004). After initial assessment of E2 and P4 concentrations over 3 months in each monkey, they were trained on two cognitive tasks and performance was evaluated in relation to hormonal concentrations. The first task assessed associative learning using a simple discrimination (SD) and behavioral flexibility (simple discrimination reversal; SDR), while the second task assessed working memory using a delayed match-to-sample (DMS) task. Based on findings suggesting improved cognition when E2 concentrations are high (Maki et al., 2002; Hatta and Nagaya, 2009), we hypothesized that a direct relationship would be revealed between learning and performance of the SD/SDR and DMS tasks and E2 concentrations, such that higher E2 concentrations (i.e., late follicular phase) would be associated with improved performance on both tasks. It is less clear how P4 concentrations would influence performance since some studies show high P4 is detrimental (Bimonte-Nelson et al., 2004) while others show high P4 (and high E2) lead to enhanced cognitive performance (Hatta and Nagaya, 2009). To examine whether phase-of-cycle influence on cognitive performance persisted following acquisition, performance was assessed for three consecutive months with re-exposure to the reversal-learning task using novel stimuli. We hypothesized that any observed differences in the SD/SDR task would dissipate in subsequent months based on previous studies that demonstrated rapid improvement on this task with repeated exposures (Kromrey et al., 2015).

MATERIALS AND METHODS

Subjects

Fourteen drug-naïve pair-housed adult female cynomolgus macaques (*Macaca fascicularis*) served as subjects (Table 1). Each monkey was fitted with an aluminum collar (Primate Products, Redwood City, California) and trained to sit in a standard primate chair (Primate Products). Monkeys were weighed weekly and feed enough fresh fruit and food (Nestle Purina PetCare Company, St. Louis, Missouri) to maintain healthy body weights as determined by physical appearance and veterinary exams; water was available *ad libitum* in the home cage which measured $0.71 \times 1.68 \times 0.84$ m (Allentown Caging Inc., Allentown,

New Jersey). All animals had a behavioral history of operant responding maintained by sucrose pellets but no drug history. A subset of these monkeys was included as a control group in a previous publication (Kromrey et al., 2015), but no approach was taken in that publication to address hormonal effects on cognition. Environmental enrichment was provided as outlined in the Institutional Animal Care and Use Committee's Non-Human Primate Environmental Enrichment Plan. All experimental procedures were performed in accordance with the 2011 National Research Council *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

Verification of hormonal fluctuation across cycle

Blood sampling occurred in 12 of the 14 monkeys (see Table 1). Two of the monkeys included in the cognitive assessments were previously on a different study and were not trained for serum collection. Monkeys were trained to sit calmly in a primate chair in a quiet room, while ~3-mL blood sample was collected from the femoral vein. Blood draws occurred every other day across three consecutive menstrual cycles. E2 and P4 concentrations were performed using a Roche Diagnostics (Indianapolis, IN) Cobas-e411 assay instrument at the Endocrine Services Laboratory at the Oregon National Primate Research Center. The assay sensitivity ranges were 5–4250 pg/ml for E2 and 0.035–59 ng/ml for P4. Intra- and inter-assay variation with the Roche Cobas-e411 is consistently less than 6% for E2 and P4. Four phases of the menstrual cycle were defined by counting backwards from menses and mean concentration of E2 and P4 during these phases were used to confirm menstrual cycle phase. These phases included early follicular (EF, menstrual cycle days 1–7), late follicular (LF, menstrual cycle days 8–14), early luteal (EL, menstrual cycle days 15–21) and late luteal (LL, menstrual cycle days 22–menstruation). Concentrations of P4 and E2 across the four-cycle phases were analyzed using separate one-way analyses of variance (ANOVA). Significant main effects were followed by post-hoc Tukey test.

Cognitive assessments

Cognitive testing was conducted 5 to 7 days per week between 9:00 am and 12:00 pm using the Cambridge Neuropsychological Test Automated Battery apparatus (CANTAB; Lafayette Instruments, Lafayette, Indiana) as described previously (Gould et al., 2012, 2013; Kromrey et al., 2015). Monkeys were first trained on the SD/SDR task and following completion of Experiment 1 (see below) trained on the DMS task with maintenance of performance on the SD/SDR assessed no more than once per week. Animals completed a maximum of 200 trials in the SD/SDR task and 80 trials in the DMS task. Only one task was assessed per behavioral session. For the SD/SDR task, total session length depended on task performance, as sessions terminated once reversal criteria were met or a maximum of 200 trials were completed (see below). In the DMS task, each animal completed 80 trials, divided up into three delay lengths (short, medium and long) which were performance based (see below), therefore the total session length varied between animals but was typically one hour.

Experiment 1. Influence of E2 and P4 concentrations on acquisition and maintenance of a reversal-learning task

SD/SDR task: Acquisition—In the SD task, three geometric shapes (A, B, C) appeared in a horizontal row across the center of the screen while a light above the food pellet dispenser was illuminated. A response on one shape (A+) resulted in delivery of a 190-mg food pellet, illumination of a light inside the pellet dispenser and a 7-second inter-trial interval (ITI) during which the monkey retrieved and ate the food pellet. During the ITI, the light above the pellet dispenser was turned off. Responding on either of the other two shapes (B–, C–) resulted in a 10-second timeout (TO), followed by a 7-second ITI. During the TO the light above the pellet dispenser remained lit but no reinforcer was delivered. Shapes were pseudo-randomly distributed throughout the three possible positions on the screen with a maximum of 200 trials per day. Acquisition of the SD was defined as 18 correct responses out of the previous 20 completed trials. Once the acquisition criterion was met, the contingencies were altered in the SDR phase so that responding on the previously correct shape was incorrect (i.e., A–) while a response on one of the previous incorrect shapes was now the correct response (i.e., B+). The third shape, which was incorrect in the SD phase, remained incorrect in the SDR phase (i.e., C–). The same consequence for responding on the correct or incorrect stimuli and the same criterion for acquisition used in the SD phase was used in the SDR phase. Since initial acquisition of the task can only occur once, the 14 monkeys were randomly distributed across the four phases for the initial exposure to the task (EF, n=3; LF, n=4; EL, n=3; LL, n=4; see Table 2).

Data Analysis—The primary dependent variables were the total number of trials to criterion, the number of errors committed, and the number of omitted trials to acquisition during the SD and SDR tasks. Response and pellet-retrieval latencies were also recorded. Perseverative errors were calculated for the SDR task, defined as responses on the stimulus that had been reinforced in the SD phase (A–). Incorrect responses on the stimulus that had not been reinforced in the SD phase (C–) were termed seeking errors. Multiple linear regressions were used to assess the relationship between E2 and P4 concentrations when acquisition occurred and the dependent variables of the reversal-learning task (i.e., total trials, errors, perseverative errors and omissions). Two-way ANOVAs were conducted using phase of cycle (EF, LF, EL, LL) and task phase (SD, SDR) as factors. Significant main effects were followed by post-hoc Fishers LSD tests. Multiple Pearson correlations were used to assess the relationship between mean E2 and P4 concentrations for the phase when acquisition occurred and the dependent variables of the reversal learning task (i.e., total trials, errors, perseverative errors and omissions). Because the data were not normally distributed, distributions were normalized with a square-root transformation to better comply with the assumptions of parametric analysis (Roberts et al., 1988; Wright et al., 2013). In all cases, differences were considered statistically significant at $p < 0.05$.

SD/SDR task: Maintenance of performance—Once all monkeys acquired the SD/SDR task, they were tested once each week for three months. On the other days of the week animals responded on the DMS task or no cognitive testing occurred. Shapes were presented in non-overlapping sets of three; the set of shapes used during each testing session was randomly selected from the “CAMCOG 0” list associated with the CANTAB system

and was not re-used throughout this experiment. Task completion criteria were identical to those described above. Two monkeys stopped cycling regularly and two others were moved to another study, therefore the number of animals that completed testing for all three months decreased to 10 monkeys. For this experiment, each monkey was tested at each of the four phases of the cycle (EF, LF, EL, LL). Blood samples were not collected during this maintenance portion of the task. The within-subject variability in sex hormone concentrations month-to-month in premenopausal subjects was not expected to be high considering previous investigations in women (Gann et al., 2001; Chatterton et al., 2005). Consistent with these reports, prior sampling of hormone concentrations in a subset of monkeys across 3 months did not significantly differ month-to-month (data not shown). Dependent variables included the total number of trials to criterion, the number of errors committed and the number of omitted trials to acquisition during the SD and SDR tasks. Three-way ANOVAs were conducted using cycle phase (EF, LF, EL, LL), stage of task (SD, SDR), and month as factors; significant main effects were followed by post-hoc Fishers LSD tests.

Experiment 2. Effects of menstrual cycle phase on DMS performance

In this task, a target image appeared on the screen and, following a response on the target, three images appeared after a 0 or 1 second delay. A response on the previously displayed image resulted in delivery of a 190-mg food pellet. A response on either of the other two images resulted in a 10-second time out and no pellet delivery. Once percent accuracy reached 80% for 3 consecutive days with this short delay, trials with gradually higher delays were included. Delay values were adjusted until task performance met predetermined criteria: short delay, >78% accuracy; middle delay, 55%–78% accuracy; long delay <55% accuracy (see Table 1 for short-, medium- and long-delay values for each monkey). Delays were randomly presented throughout each session so that there were ~27 trials per delay per session. DMS performance was deemed stable when accuracy at each delay length remained within these accuracy ranges for 5 consecutive days. Eleven of the 14 monkeys included in the analysis reached stability within 3 months of training initiation; three monkeys did not reach the stability criteria within 6 months and were therefore not included in statistical analysis (see Table 1). Once stability was reached, monkeys continued on the task at their individualized short-, medium- and long-delay values for a minimum of 3 days per menstrual cycle phase (i.e., EF, LF, EL, LL).

Data Analysis—The primary dependent variables were percent accuracy at each delay length, response latencies for target and match phases and pellet retrieval latencies. To compare baseline delay-effect curves between phases of the menstrual cycle a two-way repeated measures ANOVA with phase of cycle (EF, LF, EL, LL) and delay (short, medium, long) as factors was conducted. Significant main effects were followed by post-hoc comparisons using Fisher's LSD tests.

In both experiments, effect sizes for ANOVAs were estimated by calculating eta squared for each significant result. For pairwise comparisons, Cohen's *d* was calculated using an online calculator at <http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-SMD1.php>.

RESULTS

Baseline E2 and P4 concentrations across the menstrual cycle

Blood sampling occurred every other day across three consecutive menstrual cycles in 12 normally cycling female monkeys. Concentrations were measured and assigned a phase based on when menses was observed. A one-way repeated measures ANOVA comparing E2 concentrations across phases indicated that there was a significant main effect of menstrual cycle phase ($F_{3,36} = 31.83$; $p < 0.001$; Fig. 1, open symbols; Eta squared = 0.59). Post-hoc comparisons revealed that E2 in the LF phase of the cycle was significantly higher than concentrations from all other phases (all $p < 0.001$; Cohen's $d = 1.47$ for all); none of the other three phases were significantly different from one another. There was also a significant main effect of menstrual cycle phase on P4 concentrations ($F_{3,36} = 64.86$; $p < 0.001$; Fig. 1, closed symbols; Eta squared = 0.75). Post-hoc comparisons indicated that all phases were significantly different from one another (all $p < 0.05$; Cohen's d : for EF vs EL, LF vs EL and EL vs LL = 1.47; for EF vs LL = 1.36; for LF vs LL = 0.97) except the LF vs. EF phases.

Experiment 1. Influence of E2 and P4 concentrations on acquisition and maintenance of a reversal-learning task

SD/SDR task acquisition: E2 and P4 correlates—All the monkeys acquired the SD/SDR task within 7 days (mean 3 days $\pm$ 0.7 SEM) of initiation and therefore acquired within the same phase of the cycle as they were first exposed. Collapsed across all phases of the menstrual cycle, the mean number of total trials to meet criteria performance ($F_{1,10} = 10.86$; $p < 0.05$; Eta squared = 0.15), the number of errors ($F_{1,10} = 11.85$; $p < 0.05$; Eta squared = 0.19) and the number of omissions ($F_{1,10} = 14.49$; $p < 0.05$; Eta squared = 0.11) were significantly different in the SD compared to the SDR stage of the task (Table 2). Linear regression ($n=12$) revealed a significant positive correlation between P4 concentrations and the number of total trials ($r = 0.63$, $p < 0.05$; Fig. 2B) and errors ($r = 0.66$, $p < 0.05$; Fig. 2D) during the SD stage. In contrast, there was not a significant correlation between P4 concentrations and total trials and errors during the SDR task (Fig. 2F, 2H). E2 concentrations, while not statistically significant, were negatively associated with SD and SDR performance (Fig. 2, left panels).

While there was not a significant correlation between E2 and P4 concentrations with SDR errors, using three stimuli in the SD/SDR task allowed for the determination of whether errors made in the SDR phase were perseverative. During the SD phase, monkeys made a similar number of errors on each incorrect stimulus, indicating that, prior to reversal, no stimulus bias existed (data not shown). During the SDR (Fig. 3), a main effect of error type was found ($F_{1,10} = 26.11$; $p < 0.001$; Eta squared = 0.21) and post-hoc tests revealed a significant difference between perseverative (i.e., A–) and seeking (i.e., C–) errors for the monkeys at all phases of the cycle except the LF phase (all $p < 0.05$; Cohen's d for EF = 2.47, for EL = 1.83; for LL = 3.05). Moreover, fewer perseverative errors were made during the LF phase of cycle compared to both the EF ($p < 0.05$; Cohen's $d = 1.75$) and LL ($p < 0.05$; Cohen's $d = 1.77$) phases.

SD/SDR task maintenance—For the maintenance portion of these studies, after initial assessment of SD/SDR performance, monkeys were studied for 3 consecutive months on this task. A three-way ANOVA revealed a significant main effect of the task stage (SD vs. SDR) on the number of total trials ($F_{1,231} = 7.96$; $p < 0.01$; Eta squared = 0.03), errors ($F_{1,231} = 14.46$; $p < 0.01$; Eta squared = 0.05), and omissions ($F_{1,231} = 3.87$; $p < 0.05$; Eta squared = 0.015) to criterion (data not shown). However, the positive relationship between P4 and SD performance did not extend to this period of maintenance (data not shown).

Experiment 2. Effects of menstrual cycle phase on DMS performance

A two-way ANOVA revealed a significant main effect of delay value on performance accuracy ($F_{2,60} = 117.98$; $p < 0.001$; Eta squared = 0.71). There was no significant effect of menstrual cycle phase or an interaction between cycle phase and delay (Fig. 4). Response and pellet retrieval latencies were not significantly different across menstrual cycle phases (data not shown) nor were there significant interactions.

DISCUSSION

The goal of the present set of studies was to examine how fluctuations in estradiol and progesterone influence cognitive performance in drug-naïve, normally cycling female cynomolgus monkeys. Hormonal measures were assessed with serum concentrations of E2 and P4, which showed significant and orderly differences across the approximate 30-day menstrual cycle for three consecutive months. There was a significant relationship between P4 concentrations and learning of a simple discrimination (SD) such that monkeys with high P4 concentrations required more trials and made more errors. There was no significant relationship between E2 concentrations and SD performance. However, assessment of behavioral flexibility with the reversal task (SDR) showed enhanced performance during the late follicular phase, which coincides with high circulating E2 concentrations. These initial relationships between hormonal concentrations and cognitive performance were not maintained when monkeys were retested on the SD/SDR task over 3 months nor were differences noted in working memory as assessed with DMS.

Although some previous studies in normally cycling women did not show variation in cognitive performance across the menstrual cycle (Epting and Overman, 1998; Mordecai et al., 2008; Mihalj et al., 2014), others have demonstrated differences in working memory and verbal fluency when comparing luteal vs. follicular phases (Drake et al., 2000; Maki et al., 2002; Rosenberg and Park, 2002). The majority of prior studies tested women only twice, in the early follicular and mid-luteal phase, and observed improvements in cognitive performance were typically attributed to elevated E2 concentrations (Maki et al., 2002; Mordecai et al., 2008; Hatta and Nagaya, 2009), although measurements of E2 and P4 concentrations were not usually conducted. Furthermore, the interaction between menstrual cycle phase and cognitive performance appear to depend on the task assessed, the subject's age and for studies in ovariectomized animals, the duration of hormone deprivation (Gogos et al., 2014). For example, researchers have shown that women perform better in tasks involving verbal fluency, speed and fine motor skills during time points when E2 and P4 concentrations are high (Hampson 1990; Drake et al., 2000; Maki et al., 2002; Rosenberg

and Park, 2002; Yonker et al., 2003), whereas when concentrations of these hormones were low they performed better in tasks involving spatial ability, target-directed motor tasks and mathematical reasoning (Hampson 1990; Hausmann et al., 2000; Postma et al., 2000). One advantage of an automated system such as CANTAB is that different memory tasks (e.g., behavioral flexibility and working memory) can be studied using the same motor components, thereby eliminating a potential confounding variable.

In order to better understand the relationships between normal fluctuations in E2 and P4 concentrations in normally cycling females and task sensitivity, two different cognitive tasks were used. The SD/SDR task measure executive function and behavioral flexibility (see Gould and Nader, 2015), which are believed to be mediated through the orbitofrontal cortex, medial striatum and ventrolateral prefrontal cortex (cf. Lacreuse et al., 2014). Lacreuse et al. (2014) reported a direct relationship between E2 concentrations and poor performance. That is, exogenously administered E2 to ovariectomized monkeys resulted in more errors during the reversal phase. In the present study, while E2 concentrations were not significantly related to SD performance, there was a relationship between E2 concentrations and errors during the reversal phase (SDR) of the task, with monkeys acquiring the reversal faster in the late follicular phase when E2 concentrations are highest. The differences in results may be due to the study of normally fluctuating E2 concentrations vs. the treatment regimen utilized when E2 is administered exogenously (see Lacreuse et al., 2014 for discussion).

An advantage of studying normally cycling female monkeys is that we can assess both E2 and P4 concentrations on cognitive performance. During the follicular phase, E2 concentrations were elevated while P4 concentrations were low, and under SD conditions, low P4 was associated with enhanced cognitive performance. Thus, it could be argued that elevations in E2 concentrations do not produce cognitive enhancement, but rather luteal increases of P4 concentrations may hinder cognitive performance. We found a direct relationship between P4 concentrations and the acquisition of a simple discrimination, such that monkeys with higher P4 concentrations performed worse than those with lower concentrations. The present findings are consistent with human data where women administered a high dose of P4 performed more poorly on a delayed-recall and digit symbol substitution test (Freeman et al., 1993). Additionally, in ovariectomized rats, P4 administration resulted in compromised performance on a radial-arm maze task (Bimonte-Nelson et al., 2004). The role of P4 and memory has recently been reviewed (for review see Barros et al., 2015) and it is proposed that impairments are due to the relationship between P4 and GABAergic transmission. Specifically, certain metabolites of P4, such as allopregnanolone (3- α -hydroxy-5- α -pregnan-20-one), which have direct actions at the GABA_A receptor complex, alter the balance between excitatory and inhibitory functions of the central nervous system (Amin et al., 2006). These GABA_A receptors may also be related to the hormonal fluctuations across the menstrual or estrous cycle, since expression of these receptors is enhanced during phases when P4 peaks (for review, see Schumacher et al., 2014).

The present findings are consistent with other studies indicating that the influence of hormonal concentrations on cognitive performance is task specific (e.g., Islam et al., 2008). In fact, Barros et al. (2015) highlight the possibility that increases in P4 concentration that

occur either before or after task performance can result in either enhancement or disruption of cognition. The study of normally cycling females on tasks that required several days to achieve stable performance, as done in the present study, controls for this mediating factor. With regard to E2 concentrations and task specificity, one possibility is that E2 influences cognitive function involving tasks reliant on the PFC, through the DA system (Shansky and Lipps, 2013). It is known that DA is critical for cognitive function (Arnsten, 2011) and E2 has been shown to increase the number of DA projections from the VTA to the PFC (Kritzer and Creutz, 2008) as well as enhance extracellular DA concentrations (Xiao and Becker, 1994). Our findings that high E2 concentrations during the LF phase of the menstrual cycle did not result in significantly more perseverative responding offers indirect evidence for the involvement of DA in cognitive flexibility. The interaction of the DA system and perseverative responding is widely cited (Jentsch et al., 2002; Woicik et al., 2011; Vogel et al., 2013; Eagle et al., 2014), although to our knowledge, this is the first study to measure perseverative responding in normally cycling female monkeys.

Only one other study by Lacreuse and colleagues (2001) used direct hormonal measures to investigate whether cognitive performance fluctuated across the menstrual cycle in nonhuman primates. In that study, monkeys performed significantly better on a spatial delayed recognition span test during phases of the cycle when estrogen concentrations were low and no significant differences in a DMS task (Lacreuse et al., 2001). The lack of menstrual cycle effect on DMS performance in the present study is consistent with these results. The fact that phases when E2 concentrations are high were associated with fewer perseverative errors during the SDR task, but not working memory deficits during the DMS task may be due to different functional effects of E2 in different brain areas. For example, reversal learning is a PFC-dependent task and is highly influenced by DA signaling (Floresco and Magyar, 2006). Considering that E2 alters several aspects of the DA system (Becker, 1990; Jacobs and D'Esposito, 2011), this cognitive task may be particularly vulnerable to impairments and/or facilitation due to menstrual cycle phase. In contrast, the DMS task relies largely on the hippocampus. Although E2 receptors are found in the hippocampus (Quinlan et al., 2007), and E2 alters brain morphology and physiology in this region (Brinton et al., 2000; Woolley, 1999; Córdoba Montoya and Carrer, 1997), the functional significance of E2 in this region is yet to be fully understood. Our findings suggest that working memory as assessed with DMS may not be as susceptible to influence of natural hormonal fluctuation across the menstrual cycle as other tasks. That being stated, it should be noted that the extensive training required to establish a reliable delay curve in the DMS task (on average 10 weeks) could account for the lack of menstrual cycle effect, since training occurs across all phases of the menstrual cycle. This possibility is supported by our findings that during maintenance of the reversal task, previously observed cycle effects are no longer evident.

Although we assessed cognitive performance across the menstrual cycle, an inherent limitation of these studies is that cognitive performance takes time to assess and monkeys may be studied in more than one phase before stable cognitive performance has been achieved. We were careful to design the tasks (e.g., SD/SDR) so that performance could be assessed while monkeys remained in one phase (e.g., LF), but even then, E2 and P4 concentrations can fluctuate. For the DMS task, training necessarily occurs over multiple

cycles, making it impossible to directly assess the role of E2 and P4 on acquisition of this working memory task. In summary, our findings in normally cycling Old World monkeys indicate that P4 concentrations influence acquisition of a simple discrimination while E2 concentrations during the LF phase of the menstrual cycle appear to be associated with enhanced learning of a reversal-learning task. However, working memory, as measured by the DMS task, did not fluctuate with menstrual cycle phase. These studies add to the literature on how natural hormonal fluctuations across the menstrual cycle, particularly P4 and E2 concentrations, can have profound behavioral effects, particularly in regard to cognitive performance, that are task dependent.

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Highlights

- Cognitive function can be influenced by estradiol (E2) and progesterone (P4)
- This study examined the effects of menstrual cycle on cognition in female monkeys
- There was an inverse relationship between P4 and initial discrimination performance
- Cognition involving behavioral flexibility was enhanced in the late follicular phase
- Working memory was not influenced by menstrual cycle phase
- The findings suggest that effects of menstrual cycle on cognition are task dependent

Figure 1. Mean ($\pm$ SEM) estradiol (E2) and progesterone (P4) concentrations across the four selected phases of the menstrual cycle (n=12 monkeys per point).

Figure 2. Relationship between concentrations of estradiol (E2; left panels) and progesterone (P4; right panels) and performance on a simple discrimination (SD; panels A–D) and SD reversal (SDR; panels E–H) task. Ordinate: Number of total trials (A, B, C, D) and errors (E, F, G, H) required to meet criteria performance. Abscissa: *left*: E2 concentrations (pg/ml); *right*: P4 concentrations (ng/ml).

Figure 3.

Error distribution during acquisition of the reversal-learning task across the four phases of the menstrual cycle (n=14). Bars represent mean ($\pm$ SD) number of error responses on each non-reinforced stimulus during the SDR stage. *, $p < 0.05$.

Figure 4. Accuracy at short, medium, and long delays ($n=11$) during the four phases of the menstrual cycle. Points represent mean ($\pm$ SD).

Table 1

Subject characteristics: weight (kg), age (years), average menstrual cycle length during serum collection (days) and the individual delay times in the DMS task (seconds).

Subject	Weight	Age	Average Menstrual Cycle Length	Short	Medium	Long
C-7905	4.0	5	29.67	3	15	35
C-7902	2.8	5	27.67	3	25	50
C-7664	2.9	6	30.33	2	25	40
C-7591	3.1	6	33.33	2	15	40
C-8202	2.4	6	32.67	1	15	35
C-7558	3.0	5	28.33	3	30	45
C-7460	4.0	8	33.67	1	15	30
C-7442	2.9	8	ND	0	30	60
C-7833	2.7	10	33.33	did not reach stability		
C-7436	3.4	8	ND	did not reach stability		
C-7870	2.8	11	28.33	1	20	35
C-7889	3.8	11	27.33	did not reach stability		
C-7964	2.6	5	31	3	15	25
C-7595	3.1	5	30.33	3	15	35

ND: Not determined; blood samples were not collected

Table 2

Phase of cycle for each subject when acquisition occurred, number of total trials, errors and omissions to SD and SDR criterion.

Subject	Phase of cycle	Total Trials		Errors		Omissions	
		SD	SDR	SD	SDR	SD	SDR
C-7905	EF	101	155	58	56	0	3
C-7902	EF	48	604	10	454	1	21
C-7664	EF	28	266	9	119	0	125
C-7591	LF	59	61	13	32	0	0
C-8202	LF	72	29	3	10	48	0
C-7558	LF	25	45	7	19	1	0
C-7460	LF	57	43	24	15	3	0
C-7442	EL	32	215	12	77	0	120
C-7833	EL	120	697	91	307	0	247
C-7436	EL	112	81	36	44	0	0
C-7870	LL	45	42	13	23	0	0
C-7889	LL	236	449	79	281	3	66
C-7964	LL	40	41	13	19	0	0
C-7595	LL	314	520	154	313	50	50