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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 research has 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 four 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 acquisition of 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.

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, in press).

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. Barros 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, Barros et al. (2015) divided 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.

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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; Mantovani 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 Barros 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 was less clear how P4 concentrations would influence performance since some studies reported high P4 was detrimental (Bimonte-Nelson et al., 2004) while others showed high P4 (and high E2) lead to enhanced cognitive performance (Hatta and Nagaya, 2009). To examine whether the 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., in press).

Materials and methods

Subjects

Fourteen drug-naive 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 × 1.68 × 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., in press), 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.

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 (s).

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.

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 in 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 determined 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 to 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 tests.

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., in press). Monkeys were first trained on the SD/SDR task and following completion of Experiment 1 (see below) were trained on the DMS task with maintenance of performance on the SD/