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Influence of the natural hormonal milieu on brain and behavior in women who smoke cigarettes: Rationale and methodology

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ABSTRACT

Women experience more severe health consequences from smoking, have greater difficulty quitting, and respond less favorably to nicotine replacement therapy than men. The influence of fluctuating ovarian hormones, specifically estradiol (E) and progesterone (P), on brain and behavioral responses during exposure to smoking reminders (i.e., cues) may be a contributing factor. Results from our laboratory suggest that women in the late follicular phase of their menstrual cycle (MC) have enhanced smoking cue (SC) vulnerabilities and reduced functional connectivity in neurocircuitry underlying cognitive control, potentially placing them at greater risk for continued smoking and relapse. The primary aim of this study is to examine and link hormonal status with brain and behavioral responses to SCs over the course of three monthly MCs in naturally cycling women who are chronic cigarette smokers. This longitudinal, counterbalanced study collects brain and behavioral responses to SCs at three time points during a woman's MC. Participants complete psychological and physical examinations, biochemical hormonal verification visits, and at least three laboratory/neuroimaging scan visits. The scan visits include a 10-min SC task during blood oxygen level-dependent (BOLD) data acquisition and are timed to occur during the early follicular phase (low E and P), late follicular phase (high E, unopposed by P), and mid-luteal phase (high P, high E). The primary outcomes include brain responses to SCs (compared to non-SCs), subjective craving, E and P hormone levels, and behavioral responses to SCs. This study addresses a critical gap in our knowledge: namely, the impact of the natural hormonal milieu on brain and behavioral responses to SCs, a powerful relapse trigger. Additionally, this study will provide a roadmap for human sex differences researchers who are obliged to consider the often confounding cyclic hormonal fluctuations of women.

1. Introduction

1.1. Sex differences in cigarette smoking

Across the globe, more than 7 million deaths per year are caused by smoking [1]. A considerable body of research indicates that women experience more severe health consequences from smoking than men. Specifically, women who smoke show an increased incidence of lung cancer-related deaths, heart attacks, and chronic obstructive pulmonary disease (COPD) [1,2]. Further, women who smoke have an increased risk of pregnancy complications and perinatal mortality, including sudden infant death [3,4]. In the United States alone, close to 75% of smokers desire to quit and over half make an attempt each year [5], but success rates are low [6–8]. Compared to men, women experience greater difficulty quitting smoking [9,10] and respond less favorably to some smoking cessation treatments, such as nicotine replacement therapy [11–15]. This differential response to treatment may explain why the rate of tobacco use among men is declining; whereas, the rate among women is increasing, specifically among younger and socioeconomically challenged women [16]. Although cigarette consumption levels and dependence severity have declined in recent years in both sexes, women experience greater severity of craving, smoke to relieve negative affect at a higher rate and smoke more regularly [17]. Additionally, following overnight abstinence, women report experiencing more negative mood symptoms, greater relief from nicotine withdrawal symptoms after smoking, and greater cigarette craving compared to

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men [18]. These trends are concerning in light of decades of earlier research wherein men have continually shown higher dependence and consumed more cigarettes than women.

Given these disparities, it is critical to develop an understanding of the biological bases underlying sex differences in nicotine use disorder (NUD). A key factor underlying sex differences is ovarian sex hormones. Ovarian hormones influence basic brain chemistry during development (i.e., organizational effects) and across the lifespan (i.e., activational effects) [19]. As such, the influence of fluctuating 'activational' ovarian sex hormones on the brain, and consequently, behavior may contribute to women's greater difficulty in quitting, reduced treatment response, and increased behavioral manifestations of dependence.

The activational effects of ovarian hormones begin to exert their effects during puberty. In women, multiple hormones are secreted and participate in a cyclical and yet variable pattern, which continues throughout a woman's reproductive years. The two major ovarian sex hormones that have shown the greatest influence on female brain function and behavior are estradiol (E) and progesterone (P) [20]. These two ovarian hormones are the backbone of the female menstrual cycle.

1.2. Background on the natural hormonal milieu

Fig. 1 illustrates an idealized human menstrual cycle (MC) for women who are not pregnant and not taking exogenous hormones. A typical 28-day cycle is illustrated, consisting of two major phases, follicular and luteal, with multiple subdivisions. Menses, the time at which menstruation occurs, begins the MC. During this time of follicular development, thus termed the early follicular phase, both estradiol (E) and progesterone (P) levels are steady and low. In the late follicular phase, P levels remain low while E levels rise rapidly and transiently peak just prior to ovulation, the time at which the ovaries release an ovum. Ovulation marks the onset of the luteal phase, during which P levels rapidly rise and dominate for several days. E levels drop rapidly in the early luteal phase but do not drop to baseline. During mid-luteal phase, there is a second significant peak in E. Towards the end of the luteal phase, both hormones return to a steady and low state, completing the cycle and signaling the beginning of the next menses [20].

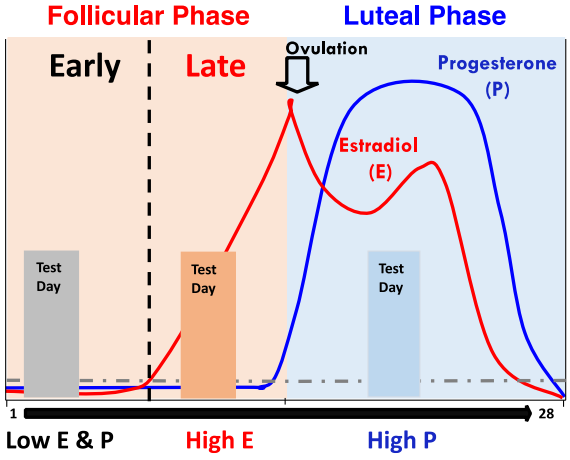

Fig. 1. An idealized menstrual cycle (MC). Diagram depicting the fluctuations of estradiol and progesterone during an idealized 28-day MC. Test Days are scheduled at strategic intervals over the course of the MC to capture the greatest variability in ovarian hormones. MRI; magnetic resonance imaging.

1.3. Research on the influence of sex hormones on nicotine addiction

Convincing research in both animals and humans suggests that E and P affect drug-related processes. Fluctuations in these hormones over the course of the estrous/MC produce direct effects on the mesolimbic dopaminergic system and affect reward-related processes in animal models of drug-associated processes. Specifically, E increases dopamine release in the ventral striatum (VS), a key component of motivational circuitry [21,22], and differentially modulates nicotine-evoked dopamine release in response to nicotine administration [23]. In animal models of relapse, E contributes to faster acquisition of drug-seeking behavior, escalation of drug consumption, and accelerated drug-primed and drug-cue induced reinstatement [24–27]. For example, rats trained to self-administer nicotine show greater levels of responding on a progressive ratio schedule, and the levels of responding inversely correlated with P and positively correlated with the E to P ratio [28].

Human research on the effects of the hormonal milieu on addiction processes such as craving, withdrawal, and relapse is limited but generally supports animal literature [29–33]. For example, Sofuoglu and colleagues (2001) have demonstrated that exogenously administered P decreased the positive subjective effects of cigarette smoking and related craving [32]. A recent study that included daily measurement of P showed that higher within-person P levels predicted reductions in the number of cigarettes smoked per day [34]. These studies and others [35,36] suggest that P, of which levels are greatest relative to E during the luteal phase of the MC, may protect against the powerful pull of reward-related stimuli, such as smoking cues. In a laboratory study of intravenous nicotine effects on a range of nicotine endpoints, DeVito et al. (2014) found a dose-by-phase interaction on nicotine-related subjective ratings with women in the follicular phase demonstrating dose-related escalating ratings of 'high', 'feel good' and 'want more' compared to women in the luteal phase [37]. Similarly, Mello (2010) observed that women in the mid-follicular phase of their cycle (compared to their mid-luteal phase) received more of a rush from smoking and experienced higher ratings of craving [31]. These studies provide initial evidence that women in the late follicular phase may experience nicotine, cigarettes, and cigarette reminders as more rewarding. Consequently, the late follicular phase may be associated with less success in quitting smoking. Indeed, two studies have shown that women who begin treatment in the follicular phase have greater difficulty remaining abstinent compared to women who begin treatment in the luteal phase [38,39]. We further suggest that quitting during the mid-luteal phase of the MC might be the time of the month when the highest success may be realized, when P is high and is exerting potential protective effects.

1.4. Smoking cue reactivity – a major relapse predictor

Several factors are involved in the motivation to smoke and the risk of relapse, including, but not limited to, stress, peer pressure, availability, low (or high) mood and weight management [40–48]. Experiencing nicotine withdrawal symptoms, including irritability, insomnia, fatigue, poor concentration, nausea, reduced appetite, and headache [49], is a major relapse predictor in early abstinence [50]. A second major relapse predictor, one that can trigger craving and relapse sometimes months or even years after quitting smoking, is exposure to smoking reminders (cues), such as the smell of a lit match, being around others who are smoking, or seeing the favored brand of cigarettes [41,42,44,47,48,51–54]. Because of its long-lasting influence on relapse, studies in our laboratory have focused on factors influencing brain and behavioral responses to smoking cues (SCs), often referred to as 'smoking cue-reactivity'.

Our preliminary neuroimaging results show enhanced responses to SCs in the medial orbitofrontal cortex, a region of the brain known to be involved in the coding of reward magnitude, in women during the fol-

licular phase (FPs) compared to women in the luteal phase (LPs) [55]. FPs, but not LPs, showed SC-elicited craving correlated with increased neural responses in the anterior ventral insula, a region consistently shown to be involved in cigarette craving [56–58]. To better understand our findings, further work in our laboratory interrogated whether MC phase influenced resting-state functional connectivity, and in turn, the relationship between resting-state functional connectivity and attentional bias to SCs. Resting-state functional connectivity examines the functional interactions between brain regions, and is thus thought to represent inherent brain organization, influencing brain function and behavior [59,60]. Attentional bias is the involuntary shift of attention to a presented stimulus; in this case, attentional bias towards SCs was assessed with a visual dot-probe attention bias task. Compared with LPs, FPs had less functional connectivity between cortical cognitive control brain regions and limbic motivational/reward regions (i.e., the dorsal anterior cingulate; and the subgenual anterior cingulate, medial orbitofrontal cortex and ventral striatum). Among FPs, but not LPs, resting-state functional connectivity between the dorsal anterior cingulate cortex and the bilateral dorsolateral prefrontal cortex was inversely correlated with attentional bias to SCs. Results suggest that FPs have reduced resting-state functional connectivity in circuits underlying cognitive control, potentially placing them at greater risk for continued smoking and relapse [61]. These cross-sectional studies were conducted in women without biochemical hormonal verification but provide preliminary supportive data towards the hypotheses guiding this study.

1.5. Limitations of the current literature

Although we have gained some insight into the effects of hormones on smoking behavior from the existing literature, much of it is limited because the variability both within and between women is not taken into account. The human MC is thought to follow a monthly pattern (see Fig. 1), but ovarian hormone concentrations and MC lengths often deviate from the archetypal 28-day flux and flow. Normal cycles, which range anywhere between 24 and 32 days, and hormone concentrations are affected by numerous external and internal influences, such as environmental stressors, exercise, health, and age. Of note, women who experience a dependable 28-day cycle every month are the exception rather than the rule. Additionally, at least one-third of all menstrual cycles are anovulatory [62]. If ovulation does not occur, the predicted monthly fluctuations in hormones are disrupted [20].

Many earlier studies used MC phase (i.e., follicular and luteal, or subphases within) as a proxy for ovarian hormones. Women were categorized based on their self-reported onset of menses without biochemical verification of hormone status. Other studies used undefined MC phase classifications, and many failed to normalize individual MCs (Review) [63]. Human studies are further confounded by the inclusion of women who are peri- or post-menopausal and women who are taking exogenous hormones (e.g., hormonal contraceptives) or other medications that affect hormone secretion. Although these early studies provide hypotheses for future work, they should be cautiously interpreted as they can lead to inaccurate and inconsistent research findings, ultimately affecting reproducibility. Biochemical verification of hormonal status, through blood/serum or saliva measures of E and P, can mitigate these issues. Indeed, a new generation of studies are emerging that use biochemical verification of hormonal status to acquire MC information [34]; however, many studies continue to be limited by categorizing participants broadly into follicular or luteal phases, rather than focusing on subphases, which are characterized by a vastly different hormonal milieu (See Fig. 1). Furthermore, studies often either provide a limited description of the methodology used to determine subphase or fail to do so completely. Given the MC and hormonal variability both within and across women, a more rigorous analysis of hormonal effects on smoking endpoints might consider grouping women by biochemically-verified MC subphase.

1.6. Study goals, relevance, and impact

In the current study, we aim to link hormonal status with brain and behavioral responses to SCs over the course of three MCs using a longitudinal counterbalanced design. Based on the emerging literature, we hypothesize that (1) women will exhibit enhanced brain (as assessed by functional magnetic resonance imaging (fMRI)) and behavioral (as assessed by a neuro-behavioral battery) responses to appetitive SCs during the late follicular phase (high E) compared to the early follicular phase (low E and P), and (2) women will exhibit reduced brain and behavioral responses during the mid-luteal phase (high P) compared to the early follicular phase. To test these hypotheses, we record brain and behavioral responses to highly appetitive SCs in a group of healthy, naturally-cycling women who are chronic cigarette smokers at three distinct time points during their natural MC. Given that the early follicular phase is associated with extremely low and steady E and P levels, it offers a natural and ideal comparator condition. Test Days are carefully timed to occur during high E, unopposed by P (HE), high P (HP), and when both E and P are low (LEP) (see Fig. 1). E and P concentrations will be used to verify conditions. We will also conduct exploratory analyses of the influence of ovarian hormone levels on day-to-day reward-related- and other behaviors using daily online surveys.

This article presents a roadmap for human sex differences researchers who are obliged to consider the often confounding cyclic hormonal variation of women in their study of the brain and behavior. Specifically, this study will provide the appropriate methodology to identify MC subphases (i.e., menses, early follicular, mid-follicular, late follicular, ovulation, early luteal, mid-luteal, and late luteal), as well as study the phenomena influenced by changes in hormones (e.g., using self-report of MC history, MC tracking, and urinary, blood (serum), and/or saliva hormone measurements). Although the current study design focuses on women who are chronic cigarette smokers, the research approach outlined below can be applied to other addictive behaviors and research fields.

2. Methods

2.1. Study objectives

We are conducting a 16-week neurobiobehavioral, daily monitoring, longitudinal study to examine the influence of ovarian hormone fluctuations on brain and behavioral responses to SCs in naturally cycling women who smoke combustible cigarettes. Participants will complete three or more hormone biochemical verification visits and three laboratory/neuroimaging sessions referred to as Test Days. Test Days will be scheduled to occur once per MC at carefully selected time points to reflect the early follicular (LEP), late follicular (HE) and mid-luteal (HP) hormonal milieus.

2.2. Recruitment

The study is being conducted at an outpatient psychiatry clinic at the University of Pennsylvania Center for Studies of Addiction. All procedures are approved by the University of Pennsylvania's Institutional Review Board (IRB No. 825716) and are conducted in accordance with the Declaration of Helsinki. All participants provide written informed consent. We aim to recruit 40 women from the Philadelphia Metropolitan Area using flyers, advertisements, mailing lists, and social media platforms (e.g., Facebook). Participant time and travel expenses are reimbursed throughout the study (see 2.3.). Currently, 21 women have been recruited who are 29.62 ± 1.33 years of age and smoke 11.57 ± 1.13 cigarettes per day, with a low to moderate nicotine dependence score of 4.40 ± 0.33 (Mean ± SEMs) [64].

2.3. Incentive structure

To minimize attrition, we implement several incentives; travel to the Center is reimbursed, compensation is provided for time, and compensation is increased for each subsequent Test Day. Additional incentives occur at the last study visit, including an end of study bonus. Payments are made with a reloadable prepaid 'credit' card that serves as a further retention incentive as participants are reminded of the benefits they receive from their study participation each time they use the card.

2.4. Inclusion and exclusion criteria

Inclusion criteria:

1. Physically and mentally healthy females, aged 18–45 years (age cutoff is to prevent the inclusion of peri-menopausal females)
2. Smoke ≥5 cigarettes/day and smoked ≥1 year prior to study start date
3. Adequate cognition and English language skills to give valid consent and to complete research assessments
4. Practice a medically acceptable, nonhormonal method of birth control, such as use of a diaphragm or condom, nonhormonal intrauterine device (IUD), or abstinence from heterosexual intercourse
5. Have daily access to the internet via computer, tablet, or smartphone

Exclusion criteria:

1. Use of nicotine products other than cigarettes, such as gums, patches, lozenges, e-cigarettes, vaping devices, or chewing tobacco
2. Current use of behavioral or pharmacological methods to quit smoking
3. Positive urine drug screen for drugs other than nicotine or cannabis
4. Diagnosis of endometriosis, polycystic ovarian syndrome (POS), or premenstrual dysphoric disorder (PMDD)
5. Current DSM-5 Axis I diagnosis (other than NUD, mild or moderate cannabis use disorder, and mild or moderate alcohol use disorder)
6. Hormonal contraceptive use during the 2 months prior to screening
7. Pregnancy, lactation, or a reported desire to become pregnant over the next four months
8. Irregular or missing menses during the three months prior to screening
9. Irregular MC length (outside of a 23–32 day window)
10. History of head trauma or injury (causing loss of consciousness lasting more than 5 min or associated with skull fracture), intracranial bleeding, or abnormal MRI
11. Magnetically-active, irremovable devices or objects in the body (e.g., intra-uterine devices, prosthetics, plates)
12. Claustrophobia or other medical condition that precludes the participant from lying in the MRI scanner

2.5. Procedures

Fig. 2 provides a general study procedures timeline. Table 1 lists the instruments and measures collected at each visit. Prospective participants are initially assessed for suitability by telephone. Interested, suitable individuals are offered in-person consent and screening.

2.5.1. Visit 1 (consent visit)

During the first visit, eligible individuals are asked to provide informed consent. They watch a video that simulates the MRI environment and then are presented with an MRI safety sheet to confirm eligibility. Finally, urine samples (to test for illicit drug use, to test for pregnancy, and to test for the presence of cotinine, the major metabolite of nicotine) are acquired.

2.5.2. Visit 2 (screening visit)

At the screening visit, a certified nurse practitioner obtains a structured medical history and performs a physical examination that includes a menstrual cycle questionnaire (MCQ; see 2.7.1). Blood samples (to probe hormonal status) and urine samples (to test for pregnancy and illicit drug use) are acquired. A trained clinician conducts a psychological evaluation, which includes the Mini-International Neuropsychiatric Interview (MINI) [65] as well as the Hamilton Anxiety (HAM-A) and Depression Scales (HAM-D) [66,67].

2.5.3. Visit 3 (baseline visit)

Eligible participants undergo a research examination scheduled to occur during the participant's LEP phase (i.e., 2–5 days after the onset of menses) to verify self-report of MC status and record baseline serum hormone levels. After a pregnancy test is performed, trained research

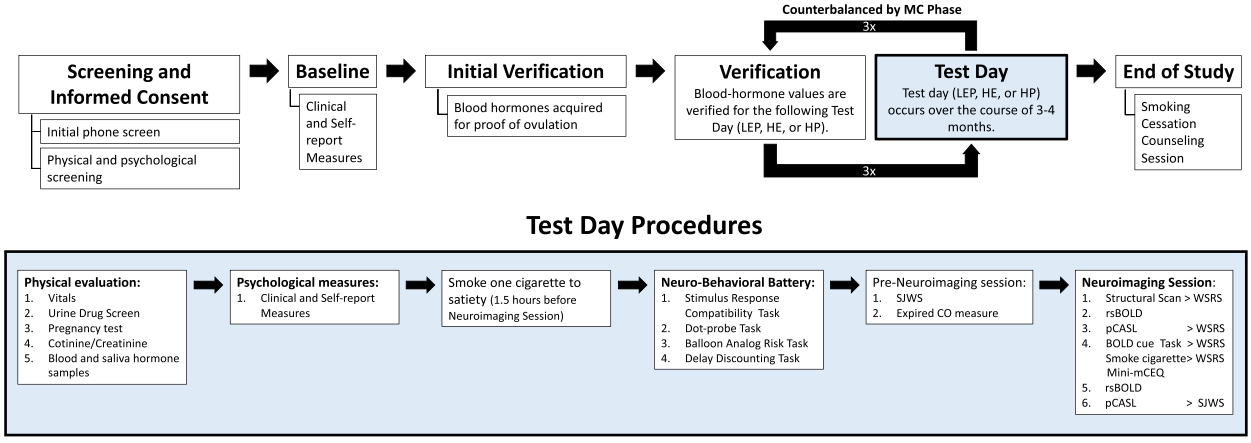

Fig. 2. Study Overview. Brief study overview with Test Day expanded. Mini-m-CEQ, Mini modified cigarette evaluation questionnaire; pCASL, pseudo-continuous arterial spin label perfusion scan; rsBOLD, resting state blood oxygen level dependent scan; SJWS, Shiffman-Jarvik Withdrawal Scale; WSRS, Within session rating scale.

Table 1
Administration time points of instruments and procedures. Measures and instruments are described in detail in Section 2.8.

Instruments/Measures	Consent	Screening	Baseline	VA 1	TD 1	VA 2	TD 2	VA 3	TD 3	EOS
Informed Consent	X									
Addiction Severity Index			X							
MRI video	X									
MRI Safety Sheet	X				X		X		X	
Expired CO measurement					X		X		X	
Urine Drug Screen	X	X			X		X		X	
Pregnancy Test	X	X	X	X	X	X	X	X	X	
Cotinine Qualitative	X									
Cotinine Quantitative					X		X		X	
E/P Blood Assay		X	X	X		X		X		
E/P/T/Cortisol Saliva Assay					X		X		X	
Medical History/Phys Exam		X								
Vitals		X			X		X		X	
Psychological Evaluation		X								
SHQ			X							
MCQ		X			X		X		X	
HAM-A/HAM-D		X			X		X		X	
RSQ			X		X		X		X	
MRI Scan					X		X		X	
WSRS					X		X		X	
SJWS					X		X		X	
mCEQ					X		X		X	
Neurobehavioral Battery					X		X		X	
Readiness to Quit										X
EOS Counseling/Referral										X
OPK (introduced at baseline visit and self-administered at home between visits)										

Note. VA = Ver Appts; TD = Test Days; EOS = End of Study; MRI = magnetic resonance imaging; CO = carbon monoxide; E = estradiol; P = progesterone; T = testosterone; SHQ = smoking history questionnaire; MCQ = menstrual cycle questionnaire; HAM-A = Hamilton anxiety scale; HAM-D = Hamilton depression scale; RSQ = reasons for smoking questionnaire; WSRS = within session rating scale; SJWS = Shiffman-Jarvik withdrawal scale; mCEQ = modified cigarette evaluation questionnaire.

staff 1) instruct participants on the appropriate use of an ovulation predictor kit (OPK); 2) advise participants on topical and/or oral medications to avoid during the study or prior to appointments due to interference with hormone assays; 3) administer the Addiction Severity Index (See 2.7.2.); 4) provide the Reasons for Smoking Questionnaire (See 2.8.8.) for the participant to complete independently; and 5) provide a thorough explanation of the daily behavioral monitoring tool, the Daily Diary (See 2.8.1.). Participants provide their first Daily Diary entry and complete a comprehensive smoking history (See 2.8.2.). Participants are then counterbalanced to one of 3 conditions (LEP, HE or HP). After this visit, trained research staff notify participants on when to begin use of their OPKs to aid in scheduling Test Days.

2.5.4. Visits 4, 6, and 8 (verification visits)

Participants provide a blood sample for assessing serum hormone levels. These E and P values are used to verify MC phase and to schedule Test Days. Once a participant completes their first verification visit and hormone levels verify ovulation, women assigned to LEP and HE will be instructed to call/text study staff the 1st day of their next menses. Women assigned to HP for their first visit will call/text when their OPK test is positive (See 2.6.1.). Test Days will be scheduled to occur within 1–4 days after the onset of menses for the LEP condition, 7–18 days after the onset of menses for the HE condition, or 5–7 days following ovulation for the HP condition. These timeframes are adjusted based on the length of each woman's natural MC. Regardless of their assigned Test Day schedule, all participants use the OPK each month to determine whether ovulation occurred.

2.5.5. Visits 5, 7, and 9 (test days)

Diurnal hormonal fluctuations, the cyclic changes in hormonal levels that occur over a 24-h period, are an important factor to consider when studying the potential influence of hormones [68]. These hormonal changes are in addition to, yet separate from, the fluctuations in hormone concentration as a function of the MC; the focal point of this study. Each Test Day procedure occurs at the same time of day across all Test Days for all participants to avoid potential confounds that may arise from diurnal hormonal fluctuations.

Test Days are scheduled to begin at 2:00 PM. On each of these visits, participants provide a urine (for drug, pregnancy, and cotinine testing), blood and saliva sample (for hormonal assay assessment). Participants complete additional Test Day measures (see Section 2.8. and presented in Fig. 1 and Table 1). Participants meet with a clinician who administers the HAM-A and HAM-D to assess anxiety and depression symptoms. At 3:30 PM, participants smoke a cigarette and then complete the 1-h Neuro-Behavioral Battery (See 2.9.). Participants begin the neuroimaging session promptly at 5:00 PM The scanning session includes a 5-min resting-state blood oxygen level-dependent (BOLD) scan, a 5-min resting-state pseudo-continuous arterial spin-label (pCASL) perfusion scan, and a 10-min SC task presented during BOLD data acquisition. Participants smoke a cigarette after the SC task, and BOLD and perfusion resting baseline scans are acquired again. Participants complete the Within Session Rating Scale (WSRS, See 2.8.5.) to assess self-reported craving, withdrawal, and mood throughout the scanning session.

Following Test Day 1 procedures, women are instructed to call/text study staff either the first day of the LH surge (positive OPK) or the first day of menses (based on their counterbalanced scan schedule) to confirm and schedule Test Day 2. This process is repeated for Test Day 3. The three Test Days are scheduled to occur over the course of 3–4 monthly MCs.

2.5.6. Visit 10 (end of study (EOS) visit)

Although the study is targeted towards non-treatment seeking smokers, research staff provide participants with information about quitting smoking, if they are interested. Participants complete a Readiness to Quit Ladder Scale (See 2.8.7.) and are offered a smoking cessation counseling session, during which local free 'Quit Smoking' resources are provided.

2.6. Biochemical measurements

2.6.1. Hormone measurements

The blood (serum) samples collected throughout the study are assayed by the Penn Fertility Care Endocrine Laboratory (same or next day). Test Day saliva samples are sent to Zava Research Testing (CLIA-certified, #38D0960950). Saliva is tested for E, P, LH, testosterone, follicle-stimulating hormone (FSH), dehydroepiandrosterone sulfate (DHEA-S), and cortisol levels. Androgens, LH, and cortisol affect brain and behavior, including cognition and reward-related processes [69,70], and as such, these measures will be used to identify factors that mediate, exacerbate, and/or ameliorate SC vulnerabilities. Evidence of ovulation is tracked using the Ovulation Predictor Kit (OPK) manufactured by ClinicalGuard [71]. This is an easy to use, qualitative test that predicts the LH surge. The level of LH in the urine is highest 24–48 h prior to ovulation. Participants are provided several individually packaged test strips at each visit. Pregnancy is assessed using FDA-approved urine pregnancy test strips that measure the presence of a hormone produced by the placenta during pregnancy, β-human chorionic gonadotropin.

2.6.2. Nicotine measurements

Expired carbon monoxide (CO), an indicator of recent smoking behavior, is measured using the MicroCo Breath CO Monitor, a calibrated CO gas-monitoring device manufactured by MicroDirect [72]. Cotinine is the major metabolite of nicotine and is an accurate measure of nicotine use. An on-site rapid qualitative nicotine test is used to detect cotinine at the consent visit. A urine quantitative cotinine screen, including the measurement of creatinine, provides a biochemical marker of smoking behavior at other time points. Samples are assayed at the Veteran's Affairs Medical Center Pathology and Laboratory Medical Services, Philadelphia, PA (CAP: 1319301).

2.7. Clinician-administered interviews and questionnaires

The Menstrual Cycle Questionnaire (MCQ) acquires information on first day of last menses, MC length, premenstrual symptoms and overall menstrual health (See supplementary material).

The Addiction Severity Index (ASI; 5th edition [73]) is a standardized, semi-structured instrument that measures multiple dimensions of substance use.

The Timeline Follow-back (TLFB [74]) assesses recent (past 30 days) alcohol, nicotine, and other substance use (quantity and frequency).

The Hamilton Anxiety and Depression Rating Scales (HAM-A, HAM-D [66,67] measure the severity of anxiety and depression symptoms, respectively.

The Mini-International Neuropsychiatric Interview (MINI; version 7.0.2) is a structured psychiatric interview used to determine DSM-5 diagnoses, including substance use disorders and psychiatric diagnoses (e.g., psychosis, acute suicidal ideation, mania, depression) [65].

2.8. Self-administered measures

Daily diary. Daily Diary data are collected and managed using REDCap (Research Electronic Data Capture) hosted at the University of Pennsylvania. REDCap is a secure, HIPPA-compliant, web-based application designed to support data capture for research studies. REDCap sends participants daily email invitations that include a link to the survey for completing their Daily Diary throughout the study (3–4 MCs). The survey takes approximately 3–4 min to complete and assesses multiple dimensions of daily behavior, such as general mood, MC symptoms, substance use (including alcohol and cannabis), sexual activity, sleep, and food consumption. To boost Daily Diary compliance, study staff maintain strong communication with participants via emails, phone calls, video conferencing, and text (See supplementary material).

The *S*moking History Questionnaire (SHQ) assesses nicotine dependence severity and acquires smoking history characteristics. The SHQ includes the Fagerstrom Test for Cigarette Dependence (FTCD; [64])

The Shiffman-Jarvik Withdrawal Scale (SJWS [75]) measures withdrawal symptoms and severity, including nicotine-related craving, general wakefulness, and physical and psychological withdrawal symptomatology.

The modified Cigarette Evaluation Questionnaire (*mCEQ* [76]) assesses the subjective effects of recent smoking. These subjective effects include smoking satisfaction, psychological reward, and aversion and craving reduction.

The *W*ithin Session Rating Scales (WSRS) is a brief measure used in our previous studies (formerly the CWQ) [56,77,78]. The WSRS consists of 9 items designed to measure state-related mood, cigarette craving, and interest in task-related stimuli. In this study, we are particularly interested in craving that is explicitly provoked by SC exposure. Thus, the WSRS is administered upon entering the scanner as a baseline measure, again prior to the SC task, and immediately following SC exposure to obtain a SC-induced change score. A final WSRS is administered after smoking to determine if and how smoking altered subjective craving.

The Questionnaire of Smoking Urges (QSU [79]) is used to assess motivations for smoking cigarettes (e.g., to reduce stress, SC exposure, to reduce withdrawal symptoms).

The Readiness to Quit Ladder Scale measures the participant's motivation to quit smoking during the final study visit. It is a single-choice scale, ranging from 1 to 10, wherein a higher score indicates greater enthusiasm to quit smoking cigarettes [80].

The Reasons for Smoking Questionnaire is a 34 item, 4-point Likert-scale, self-report measure that probes fundamental motivations for smoking. Individual reasons underlying smoking behavior (e.g., to reduce WD symptoms, SC exposure, stress) may be helpful in planning personalized smoking cessation strategies.

2.9. ***The Neuro-behavioral Battery (NBB)***

2.9.1. Overview

The tasks described below are completed within a 1-h session. Participants sit in front of an eye-level computer screen, are provided specific instructions for each task, and respond by pressing specified computer keys. To ensure appropriate engagement, study staff monitor participants from an adjoining room via a wireless camera.

2.9.2. Stimulus-Response Compatibility Task (SRCT)

The SRCT measures automatic “approach” and “avoid” tendencies toward or away from a stimulus (e.g., cigarette-related) We aim to investigate whether, and if so, how brain regions and hormones facilitate one's “approach” and “avoid” tendencies when presented with cigarette-related stimuli. In this task, the response time (in msec) to move a manikin towards or away from smoking-related and matched control pictures is measured. The average response time to approach smoking-related and avoid control images (approach-smoking) is compared to the average speed to avoid smoking-related and approach control images (avoid-smoking). This task is administered using Inquisit software [81].

2.9.3. Attentional Bias Task (ABT)

The dot-probe attentional bias task measures the salience of a picture (e.g., smoking-related). Participants’ reaction time (in msec) to correctly identify a target (an asterisk) placed in the same location as a previous target (following a smoking-related or control image) across trials [82,83] is measured. When used as a regressor in an fMRI drug cue task analysis, the attentional bias score can reveal brain regions

that respond to the incentive value of the cues. For example, we have shown that attentional bias scores in a cue vulnerable sub-group were positively correlated with amygdala/hippocampal responses to SCs [83]. This task is administered using E-Prime (version 3.0, Psychology Software Tools, Inc.).

2.9.4. Balloon Analog Risk Task (BART)

The BART provides an ecologically valid model to assess human risk-taking propensity and behavior [84]. Since risky behavior is associated with hormone levels [85], we aim to identify brain regions associated with both the changes in hormone concentrations and risk-taking behavior in a substance-using population. During the task, participants inflate a virtual balloon that either increases in size (gaining monetary reward for each pump) or explodes (loss of monetary reward). The task consists of 60 trials (balloons) with a minimum of 1 and a maximum of 24 pumps each. The primary outcome measure is the adjusted average number of pumps, which is the average number of times that the participant pumped the balloon during a session – only considering unexploded balloons. This task is administered using E-Prime (version 3.0, Psychology Software Tools, Inc.).

2.9.5. Delay Discounting Task (DDT)

The DDT measures the subjective rate at which one discounts delayed rewards (described in terms of a *k* value), which is thought to be associated with impulsivity or "loss-of-control" in drug-dependent individuals [86,87]. Because estradiol is found to influence discounting behavior [88], we aim to further investigate brain regions associated with changes in hormone concentrations and impulsivity. In the task, participants choose between smaller immediately available hypothetical monetary rewards and larger rewards available after a delay (in days). Over the course of 51 trials, the magnitudes of the sooner and later rewards and time of delay varies across trials. This task is administered using E-Prime (version 3.0, Psychology Software Tools, Inc.).

2.9.6. Stop-Signal Task (SST)

The SST offers a measure of cognitive control, which is a process shown to be affected by both MC phase [90] and addiction status [89]. Thus, we aim to include the SST score when analyzing the fMRI drug cue task data to understand how brain regions pertinent to inhibition may be influenced by hormonal status. The SST requires participants to respond as quickly as possible when a 'go' target (left or right arrow) appears by hitting the corresponding left or right button on the computer keypad. Participants are instructed to inhibit responses when a 'stop' signal (a red vertical arrow) occurs. Each run consists of 80 'go' trials and 40 'stop' trials. The delay to presentation of the stop signal following the go signal is adjusted until the subject can successfully inhibit the response on 50% of trials. The final mean delay of the stop signal, based on this 50% success rate criterion, is subtracted from the mean go reaction time, providing the stop signal reaction time (SSRT), which is the behavioral measure of response inhibition. This task is administered using software provided by Verbruggen, Logan, and Stevens (2008) [91].

2.10. SC and nonSC stimuli and Cue Rask

The smoking and nonsmoking cues for this task were designed specifically for the sample we intend to study. SCs are comprised of people 20–40 years of age and of various ethnicities who are displaying behavior indicative of 'enjoyment' while smoking cigarettes. Some of the images include rituals associated with smoking, such as 'packing' the cigarettes or lighting a match to the tip of the cigarette. Comparator (non-smoking) cues consist of similar, but different, actors engaged in activities, such as driving, crocheting, or folding hand towels (See Fig. 3). Our cue paradigm is 10 min in length and consists of 20 10-s fixation cross screens (a black screen with an X at its center) interspersed with 10 of each 20-s smoking and non-smoking cues in a fixed order. Background crowd noise and generic instrumental music are included in both sets of cues but not in fixation screens. Stimuli are presented using E-prime (version 3.0, Psychology Software Tools Inc.)

In a longitudinal study, one must consider the effects of habituation on cue reactivity. We developed three different cue sets that are similarly valenced and of equivalent salience to control for possible habituation effects. Test day scheduling and cue sets are counterbalanced by MC phase to control for order effects.

2.11. Imaging Data Acquisition

Magnetic resonance imaging (MRI) scanning is conducted on a Siemens MAGNETOM Prisma 3.0 T Trio whole-body scanner (Siemens AG, Erlangen, Germany) using a 64-channel head coil. To co-register functional and perfusion data, a T1-weighted three dimensional high resolution magnetization-prepared rapid acquisition with gradient echo (MPRAGE) scan is acquired (repetition time (TR)/echo time (TE)/inversion time (TI) = 1820/3.51/1100 ms, flip angle = 9°, band-

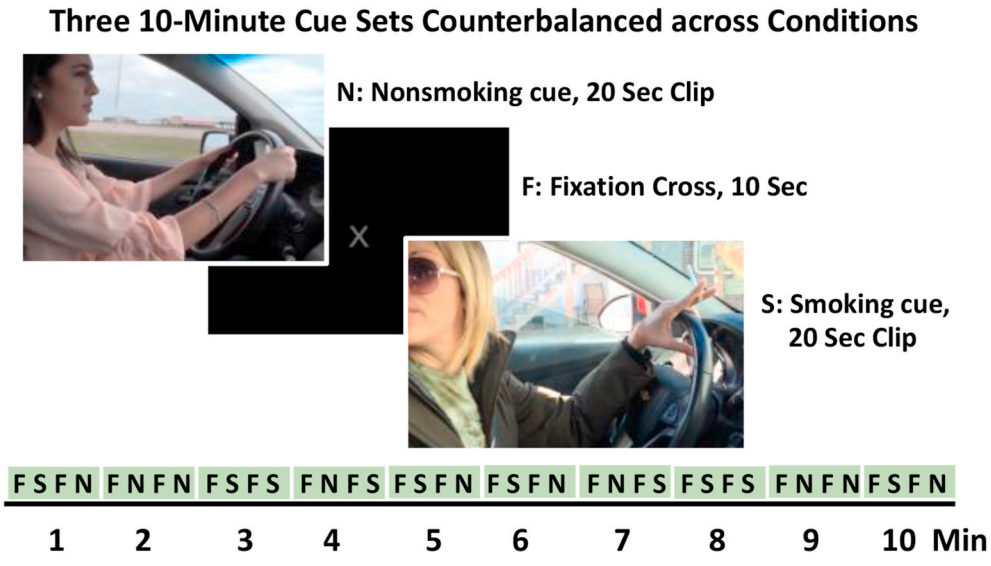

Fig. 3. Imaging session cue task. Representation of the task structure and the smoking and nonsmoking stimuli utilized during the BOLD cue reactivity task. Each 1-min bin begins with a fixation cross. Each 10-min cue set contains 10 of each 20-s smoking and nonsmoking video clips.

width = 130 HZ/Px, voxel size = 0.9 x 0.9 × 1 mm, matrix = 192 × 256, slices = 160, slice thickness = 1 mm, field of view (FOV) = 240 mm). Resting state BOLD functional data are acquired using gradient-echo planar imaging (EPI) (slices = 72, slice thickness = 2 mm, matrix = 64 × 64 × 18, flip angle = 52°, TR = 800 ms, TE = 37 ms, voxel size = 2 × 2 × 2 mm, FOV = 208 mm). Three-dimensional pseudo continuous arterial spin label (pCASL) perfusion fMRI sequence is used to acquire resting perfusion baseline scans (labeling time = 1800 ms, post-labeling delay = 1800 ms, matrix = 64 × 64 × 18, flip angle, 90°, TR = 4500 ms, TE, 10 ms, slice thickness = 3.75 mm, voxel size = 3.8 × 3.8 × 3.8 mm). Parameters for the BOLD functional data acquired during the Cue Task are: slices = 75, matrix = 64 × 64 × 18, flip angle = 70°, TR = 2000 ms, TE = 30 ms, slice thickness = 2 mm, voxel size = 2 × 2 × 2 mm.

2.12. Analyses

2.12.1. Behavioral Data Analyses

Statistical significance tests will use an alpha of .05. Continuous variables (e.g., age, education, FTCD, QSU, SJWS, mCEQ, and the WSRS composite scores) are checked for normality, transformed if necessary, and summarized by calculating means, standard deviations, and ranges. Relevant measures will be included as covariates of interest when conducting analyses. Nominal variables (e.g., race) will be summarized by calculating proportions. When appropriate, comparisons across conditions will be conducted using a one-way repeated measures ANOVA. Demographic (e.g., age and years of education) and clinical (e.g., depression and anxiety) variables will also be summarized and compared across conditions using t-tests and ANOVAs, when appropriate. Variables that differ by hormonal status will be included as variables of interest in brain and behavioral analyses. For example, anxiety and stress have been identified as key factors in smoking-related behavior and cue reactivity, especially for women [92,93]. As such, mood measures that are acquired at 4 or more time points will be examined for differences.

2.12.2. Hormonal Analyses

One-way repeated measures ANOVAs will be used to analyze serum and saliva ovarian hormone (E and P) concentrations and E to P ratios, which have been shown to correlate with addictive behavior [28]. Post-hoc pairwise comparisons using t-tests will be Bonferroni corrected. Means, SEMs, and ranges of the ovarian hormones for each subphase and measurement type (saliva or serum) will be summarized.

2.12.3. Neurobehavioral Battery (NBB) Analyses

NBB analyses will follow procedures outlined in our previously published papers and by those of our collaborators: Attentional Bias Task, [83]; Balloon Analog Risk Task, [84]; Delay Discounting Task, [86]; the Stimulus-Response Compatibility Task [94]; and the Stop-Signal Task [89,91]. The data from each task for each test day will be quantified and subjected to a one-way repeated measures ANOVA with the quantitative task data as the dependent variable and subphase as the independent variable.

2.12.4. Imaging Data Analyses

A multitude of analyses will be conducted on several scans per imaging session and behavioral variables acquired. Procedures will follow our previously published analysis techniques, but advances and optimizations will be included as we progress [61,83,95,96]. Data analyses are conducted using statistical parametric mapping software (SPM, version 12) on a MATLAB platform (2018).

3. Results

Data acquisition is underway. Here, we present results from preliminary hormonal data analyses to show feasibility of acquiring specific MC subphase data on brain and behavior (Fig. 4). As previously discussed, MC length varies both within and between women. As such, each participant's MC was normalized to the average MC length across all participants (M = 28.62 days, SEM = 0.42). This was accomplished by dividing the day of the participant's MC, wherein hormonal assays were acquired, by the length of that specific MC, multiplied by the average MC length of all the study participants. Fig. 4 depicts the normalized days for women recruited thus far, for both serum and saliva-derived values.

A one-way repeated measures ANOVA showed a significant effect of subphase on saliva E concentrations, $F(1, 18) = 12.57$, $p = 0.002$, serum E concentrations, $F(1, 14) = 11.05$, $p = 0.005$, saliva P concentrations, $F(1, 18) = 156.24$, $p < 0.001$, and serum P concentrations, $F(1, 14) = 83.69$, $p < 0.001$.

Post-hoc pairwise comparisons using t-tests with Bonferroni correction indicated that saliva E levels were significantly higher during HE (M = 0.99, SEM = 0.06) compared to LEP (M = 0.56, SEM = 0.02) as expected ($p < 0.001$). There was no significant difference in E concentration between HE and HP (M = 0.89, SEM = 0.07; $p > 0.05$). Saliva E levels were significantly higher during HP than during LEP ($p < 0.001$). As illustrated in Fig. 4, serum E concentrations followed the same pattern. Levels were significantly higher during HE compared

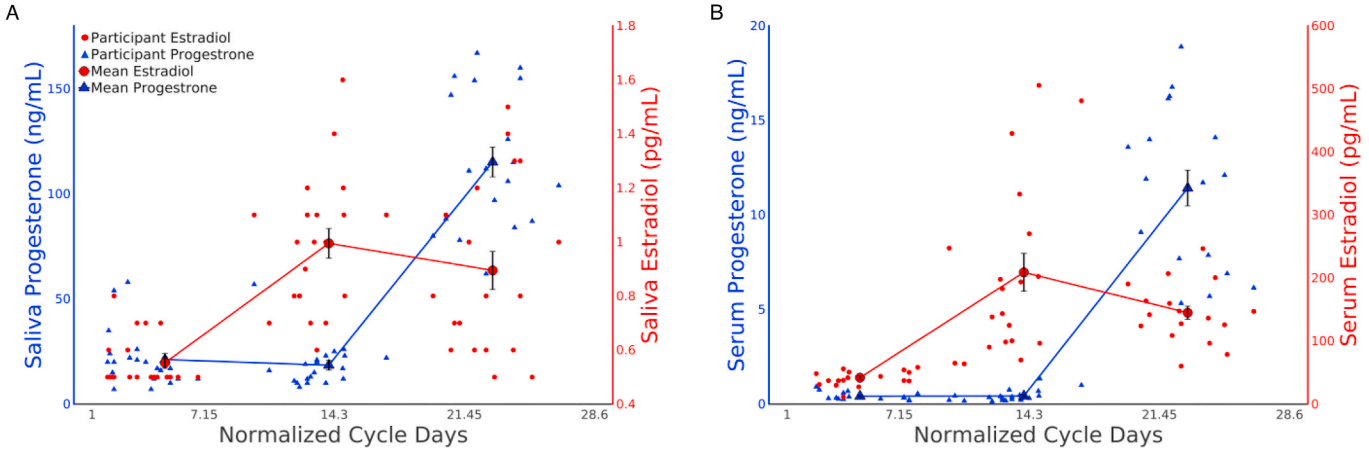

Fig. 4. Saliva and serum concentrations of estradiol and progesterone. The saliva (A.) and serum (B.) estradiol and progesterone levels as a function of the (normalized) menstrual cycle (MC), showing feasibility of acquiring hormones during select MC subphases. Values are recorded as either baseline LEP subphase or specific to a Test Day. Larger triangles and circles superimposed on the scatter plot indicate mean saliva and serum hormone concentrations during each MC subphase. Error bars indicate standard error of the mean.

to LEP (M = 208.95, SEM = 31.51 and M = 41.9, SEM = 3.06, respectively; $p = 0.001$), and there was no significant difference in E concentration between HE and HP (M = 144.92, SEM = 11.56; $p > 0.05$). Additionally, serum E levels were significantly higher during HP than during LEP ($p < 0.001$).

Saliva P concentrations remain relatively constant during LEP and HE (M = 21.74, SEM = 3.18 and M = 18.37, SEM = 2.51, respectively, $p > 0.05$), and were significantly highest during HP (M = 115.21, SEM = 7.47) compared to LEP ($p < 0.001$) and HE ($p < 0.001$). Similarly, serum P concentrations peak during HP (M = 11.42, SEM = 1.05) compared to LEP (M = 0.41, SEM = 0.05; $p < 0.001$) and HE (M = 0.43, SEM = 0.07; $p < 0.001$), while levels between HE and LEP remain low and steady ($p > 0.05$).

Results demonstrate the feasibility of using hormone concentrations to clearly identify MC subphase. We find that the mean fluctuations in ovarian hormones for both saliva and serum correspond well with the literature [97] and are qualitatively similar to each other. However, as shown in Fig. 4, the saliva hormone range is limited. Specifically, the possible saliva E values fall between 0.5 and 2 (pg/mL) in increments of 0.1. The assay that we used denotes any value less than 0.5 as "≤ 0.5". Alternatively, serum E values fell between 11.41 and 505.5 (pg/mL), providing a more granular representation of E, which allows us to better identify the relationship between ovarian hormones and other phenomena when conducting correlational analyses.

4. Discussion

4.1. Overview

This study will evaluate the impact of the natural hormonal milieu on brain and behavioral responses to appetitive SCs. A wealth of additional knowledge regarding the influence of hormones on brain and behavior will also be attained by acquiring several additional brain and behavioral measures. The study results have the potential to vastly improve our understanding of the influence of hormones on addictive processes and may show that simply timing Quit Smoking attempts to select times within a woman's MC could improve smoking cessation success. The experimental design provides an exemplar for guiding protocol development of longitudinal studies investigating the effects of hormones on brain and behavior.

MRI is a powerful tool to examine neural mechanisms underlying behavior and has been used extensively to gain insight into brain vulnerabilities that mediate maladaptive responses to drug cues. Using fMRI, brain responses during SC exposure have been shown to predict the ability to maintain abstinence or relapse to smoking in treatment-seeking women [51]; thus, this objective tool can provide clinically meaningful knowledge on the neurobiology underlying relapse. Using our neuroimaging cue paradigm, we have consistently shown robust brain responses to SCs in reward-related mesocorticolimbic circuitry (medial orbitofrontal cortex (mOFC), VS, ventral pallidum (VP), hippocampus, amygdala, and anterior ventral insula), with the most consistent findings occurring in the mOFC and VS [56,77,78]. These findings are in agreement with a substantial literature examining brain regions involved in processing reward and motivation [98–100].

Only two studies have used fMRI to study the effects of MC and/or ovarian hormones on SC exposure. The two studies are in relative agreement: A pilot study (13 completers, not prospectively randomized by MC phase) found that SC reactivity was greater during the follicular phase condition in relevant brain regions, although direct comparisons between conditions failed to reach significance [101]. Further, it is unclear whether the smoking-related images from the study elicited a desire/craving to smoke as craving was not measured post-cue exposure. The second SC study, conducted by our group and described in the introduction, found that women tested during the follicular phase had increased neural responses to SCs in the mOFC and showed brain correlates of SC-elicited craving, whereas women tested during the luteal phase had neither. This preliminary cross-sectional study was conducted in women without biochemical hormonal verification and without tasks to probe SC behavioral biases and/or reward function but provides preliminary supportive data towards our hypothesis [55]. Collectively, the available data suggest the mOFC, a region known to be involved in the coding of reward magnitude [102,103], is a reasonable *a priori* region of interest to explore differential effects of the natural hormonal milieu on SC responses. Additionally, we will expand our exploration to other players within the cortico-limbic system, including the dorsolateral prefrontal cortex, given its potential role in cognitive control over goal-directed motivated processes [61]; and the putamen, a potentially novel target [104].

Although interesting and informative, brain responses to drug cues provide limited information on relapse vulnerabilities. Because of a significant overlap in the brain processes of appetitive and aversive motivation [105–107], drug cues can neither predict direction of motivated behavior nor reveal affective valence. The innovation of the current study is that we will link neural responses to SCs to implicit behavioral responses that provide information on salience and valence providing useful behavioral proxies for relapse vulnerability.

4.2. Lessons Learned

The current investigation uses a multitude of tools to align participant hormonal status with Test Days and the hormonal "essence" of each phase (LEP, HE, and HP) during a natural MC. Throughout the study, staff manages a subject-specific calendar, which tracks the participant's first day of menses, OPK results, length of MC, and verification visit hormone levels. For the first Test Day, an average participant MC length is used to identify when to begin OPK testing. This information is estimated or ideally, derived from "period tracker" apps. After a participant receives a positive OPK, indicating that ovulation occurred, a 'tentative' Verification visit and Test Day are confirmed on the participant's calendar. If qualifying factors (i.e., began menses) are met and verify the phase that matches the participant's counterbalanced schedule, then the Test Day is updated from 'tentative' to 'scheduled.' On Test Days, E and P serum hormones are acquired as they provide immediate information on MC phase. Saliva hormones are also acquired on Test Day in order to obtain values for the full hormonal milieu (e.g., E, P, testosterone, cortisol, etc.). Saliva-based assays are also performed and provide hormone values from the bioavailable fraction, which may more accurately reflect the biological effects exerted by the hormone [97]. A discourse of the pros and cons of saliva versus serum assays are beyond the scope of the focus of the current study, but the decision on which to use is an important one. In our design, we felt it prudent to collect both based on our hypotheses. Because we wanted to control for any potential confounds from other hormones, we began our study acquiring saliva-based hormone measures for our Test Day data. Unfortunately, the estradiol results did not meet our expectations despite the test being minimally invasive, lending to their practicality over serum. Saliva-based assays for cortisol, progesterone, and testosterone compare remarkably well with serum levels; however, estradiol is technically difficult to measure in saliva, and not all labs are capable [97]. Although the lab we use to analyze our saliva samples does assay estradiol, a detection threshold of 0.5 pg/mL is used, which is above the level of many of our LEP samples. Thus, if one's hypothesis only required the identification of phase, this test would be optimal, but it is less than optimal if values are to be used as a continuous variable (See Fig. 4).

In order to schedule study visits, the research team must obtain specific and accurate details about a participant's MC (i.e., first day of last menstruation, MC length). The research staff ask participants to provide these details, and in doing so, we found that 65% of women who consented used a period tracker app (e.g., Flo Period tracker™, Ovulation

& Pregnancy tracker™). Period tracker apps collect MC details from prior MCs and provide strong predictions of future menses' as well as reliable estimations of one's average MC length [108]. This relatively new development in MC tracking allows research personnel to more accurately coordinate a participant's involvement in the study. For example, the period tracker apps help in identifying an opportune time to begin OPK testing and coordinate Test Days.

4.3. Strengths and Limitations

The longitudinal within-subjects counterbalanced design is a strength of the study, as is the choice of our comparator condition. In past studies investigating the influence of the hormonal milieu on smoking behavior, the comparator group is often either nonexistent or men [35,93]. In this study, we use the LEP phase as the natural and ideal comparator condition, given it is associated with extremely low levels of both E and P (See Figs. 1 and 4). Further, this additional repeated measure strengthens the study design by reducing the natural variance across individuals. Another strength of the study is our use of three similarly valenced sets of SCs that are presented over three MCs (temporally separated). Finally, implementing a daily diary in congruence with Test Day and verification measures poses a particular strength due to its scope and relevance. For example, this tool would allow us to further understand how mood, craving, and ovarian hormones are interrelated, providing additional insight into potential relapse factors. However, such an analysis would not probe at the relevance of more severe affective vulnerabilities, such as PMDD or those from the DSM-5 Axis I, as they may be confounding variables for the broader focus of our study. Such a limitation offers an opportunity for future research.

Although the research design has several strengths, there are limitations to consider. One such limitation is the lack of daily hormone monitoring. Daily hormone monitoring would allow a more granular understanding of a participant's hormonal status and pair well with the daily behavioral monitoring. It is feasible to implement such a micro-longitudinal approach by using daily dried blood spot testing, which women perform at home. Blood spot assays are a minimally invasive, sensitive, reliable, and accurate measure of circulating gonadal hormones [109,110]. Importantly, women do not find them overly burdensome [111]. Unfortunately, adding this daily measurement to our procedures is cost-prohibitive. Nonetheless, our hormone assay collection timepoints are timed to occur at the same time of day on each Test Day, allowing us to sufficiently test our primary hypotheses. A second limitation is the lack of a fourth Test Day scheduled to occur during the premenstrual phase (1–3 days prior to menses). The premenstrual phase is a crucial time for women who smoke cigarettes, as it is associated with increased craving and withdrawal (Review) [112]. We strongly considered including this fourth Test Day in our design but decided against it for reasons of increased subject burden and associated costs.

5. Summary

Women experience greater health consequences from smoking yet respond less favorably to available treatments. Thus, there is a crucial need to expand our knowledge regarding the brain and behavioral vulnerabilities for continued smoking and relapse in women in order to provide targeted and personalized treatment programs. The results of this study will add to the current literature examining sex differences and hormonal influences on the brain and behavior. By biochemically verifying hormone levels and examining brain and behavior at three distinct time points across multiple MCs, this will be the first study to accurately and precisely link ovarian hormone status to brain and behavior. As such, this study has the potential to help design future sex differences and ovarian hormone studies and to facilitate the development of hormonally-informed treatment approaches.

CRediT authorship contribution statement

Reagan R. Wetherill: Writing – original draft, Writing – review & editing, Supervision, Conceptualization, Methodology, Visualization, Funding acquisition. **Nathaniel H. Spilka:** Writing – original draft, Writing – review & editing, Methodology, Formal analysis, Investigation, Data curation. **Melanie Maron:** Writing – review & editing, Supervision, Project administration, Conceptualization, Investigation, Data curation. **Heather Keyser:** Writing – review & editing, Investigation. **Kanchana Jagannathan:** Writing – review & editing, Formal analysis, Data curation. **Alice V. Ely:** Writing – review & editing, Formal analysis. **Teresa R. Franklin:** Writing – original draft, Writing – review & editing, Supervision, Conceptualization, Methodology, Visualization, Funding acquisition.

Declaration of competing interest

The authors declare no financial interests or conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.conctc.2021.100738.

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