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Influence of Menstrual Cycle Phase on Neural and Craving Responses to Appetitive Smoking Cues in Naturally Cycling Females

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Abstract

Introduction: Functional magnetic resonance imaging (fMRI) has been used extensively in an attempt to understand brain vulnerabilities that mediate maladaptive responses to drug cues. Using perfusion fMRI, we have consistently shown reward-related activation (medial orbitofrontal cortex/ventral striatum) to smoking cues (SCs). Because preclinical and clinical studies generally show that progesterone may reduce reward and craving, we hypothesized that females in the follicular phase of the cycle (FPs; when progesterone levels are low) would have greater reward-related neural responses to SCs compared with females in the luteal phase (LPs).

Methods: Sated cigarette-dependent premenopausal naturally cycling females underwent pseudo-continuous arterial spin-labeled perfusion fMRI during exposure to 10-min audio visual clips of appetitive SCs and non-SCs. Brain responses to SCs relative to non-SCs were examined among females grouped according to menstrual cycle (MC) phase at the time of scanning (22 FPs, 15 LPs). Craving scores were acquired pre- and post-SC exposure.

Results: FPs showed increased neural responses to SCs compared with non-SCs in the medial orbitofrontal cortex ($p \leq .05_{corrected}$), whereas LPs did not. FPs reported SC-elicited craving ($p \leq .005$), whereas LPs did not. Within FPs, SC-induced craving correlated with increased neural responses in the anterior insula ($r = 0.73$, $p < .0001$).

Conclusions: FPs may be more vulnerable to relapse during appetitive SC exposure than LPs. Because the influence of MC phase on drug cue neural activity has not been examined, these results contribute to our knowledge of the neurobiological underpinnings of responses to drug cues, and they highlight the importance of monitoring menstrual cycle phase in all areas of addiction research.

Introduction

The devastating health consequences of cigarette smoking underscore the importance of identifying relapse predictors and implementing strategies to increase cessation success rates in both sexes, however it may be of even greater importance for women. The health consequences are more severe for females and extend to their unborn children.[1] Ironically, several studies have shown that females have

more difficulty quitting smoking than males[2,3] and available therapies are less effective in females.[4,5] There is a crucial need to advance our understanding of the neurobiology underlying the differences in smoking behavior between males and females so that treatment strategies can be tailored to improve relapse rates for both sexes.

Multiple preclinical studies have shown that female rats show a greater propensity to self administer drugs of abuse[6,7] and that this proclivity is modulated by the neuroactive steroid hormones, progesterone and estradiol[7–11] cf.[12] suggesting that relapse vulnerabilities may vary across the menstrual cycle (MC) phase. In particular, in rats trained to self-administer nicotine, levels of responding on a progressive ratio schedule were inversely correlated with progesterone and positively correlated with the estradiol to progesterone ratio.[10] Modulation of nicotine-related behaviors by the hormonal milieu has been studied quite extensively in human trials.[13–16] For example, Sofuoglu and colleagues[15] have demonstrated that exogenously administered progesterone decreased the positive subjective effects of cigarette smoking and related craving.[15] This study and others[17,18] suggest that progesterone, of which levels are greatest relative to estradiol during the premenstrual or luteal phase of the MC, is protective. In support, in a laboratory study of intravenous nicotine effects on a broad range of nicotine endpoints, DeVito et al.[19] found a dose-by-phase interaction on nicotine-related subjective ratings with females in the follicular phase demonstrating more dose-related changes and having elevated ratings of "high," "feel good," and "want more" compared to females in the luteal phase.[19]

Although the literature is somewhat conflicted, one might posit that females in the follicular phase, which corresponds to the phase of the MC when progesterone is at its lowest concentration, may experience nicotine and smoking reminders (i.e., smoking cues) as more rewarding and thus this phase may be associated with less success in quitting smoking. Indeed, two studies have shown that females who begin treatment in the follicular phase (FPs) have greater difficulty remaining abstinent compared to females who begin treatment in the luteal phase (LPs).[20,21] Interestingly, FPs faired better in treatment than LPs when nicotine replacement therapy (NRT) was provided.[22,23] Franklin and Allen[24] suggest that protection from withdrawal symptoms provided by NRT early in treatment prevented a lapse from occurring in the FPs when females experience more reward from smoking, and thus they were able to remain abstinent during their most vulnerable time.[24] In support of the hypothesis that FPs experience greater reward from smoking, Mello[14] observed that females in the follicular phase of their cycle (compared to their luteal phase) received more of a rush from smoking that was followed by higher ratings of craving.[14]

Both the nicotine present in tobacco and the stimuli associated with smoking behavior lead to relapse.[25] Indeed, even years after quitting smoking, environmental or internal reminders to smoke provoke relapse. Thus, the field has substantial interest in understanding the biology underlying smoking cue (SC) reactivity. SCs elicit craving and changes in various physiological measures such as heart rate, blood pressure, and skin conductance.[26,27] Studies examining the impact of sex on physiological and subjective responses to SCs report inconsistent findings. In some studies, females reported higher SC-induced craving and showed greater changes in mean arterial pressure than males,[28,29] yet others have shown that males had increased skin temperature responses and higher blood pressure to SCs than females.[30] Some studies reported no sex differences in physiological or subjective reports of craving.[31] While these inconsistent findings may be related to methodological differences between studies, they may also be related to the influence of MC phase on smoking behavior.[22] For example, while no effect of sex was observed on SC responses in a laboratory paradigm that included watching a video that contained smoking reminders, an effect of MC phase was observed.[32]

Functional magnetic resonance imaging (fMRI) is a powerful tool to examine drug cue reactivity and has been used extensively in an attempt to understand 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,[33] thus this objective tool can provide clinically meaningful knowledge on the neurobiology underlying relapse. Our laboratory uses *pseudo*-continuous arterial spin-labeled (*p*CASL) fMRI to study SC reactivity. Within our neuroimaging paradigm we have consistently shown robust brain responses to SCs in reward-related mesocorticolimbic circuitry (medial orbitofrontal cortex [mOFC], ventral striatum/ventral pallidum, hippocampus, amygdala, and insula) with the most persistent findings occurring in the mOFC and ventral striatum.[34–36] These findings are in agreement with a substantial literature.[37–39] Recently, we examined sex differences in SC relative to non-SC exposure and reported that both sexes had robust responses in the mOFC with males also exhibiting increased ventral striatum/ventral pallidum responses. Direct comparisons between male and female brain responses revealed that males showed greater bilateral hippocampal/amygdala activation to SCs relative to non-SCs.[40] One limitation of that study was insufficient sample size to test hypotheses regarding the influence of MC phase on SC reactivity, which we are now positioned to test. Given that preclinical and clinical studies generally show that lower concentrations of progesterone may exert protective effects, we hypothesized that FPs (compared to LPs), would show greater responses to SCs in reward-related circuitry, specifically in regions where we consistently observe SC responses in our paradigm. Based on our previous research described above, our *a priori* regions include the ventral striatum, ventral pallidum, mOFC, insula, amygdala, and hippocampus. To this end, we used *p*CASL perfusion fMRI to acquire brain responses during SC versus non-SC exposure in premenopausal naturally cycling females separated by MC phase. To date, no studies have examined the effect of MC phase on drug cue-induced neural responses.

Methods

Participants

Participants were recruited from the Philadelphia area via a variety of venues for an ongoing study examining individual differences in SC reactivity and other smoking behavior. All eligible and interested participants provided informed consent prior to enrollment. Telephone screens and medical and psychiatric evaluations were used to determine participant eligibility. Ineligible participants were those who reported substance abuse disorders within the last six months, had current Axis I DSM IV psychiatric diagnoses, had significant medical conditions, took medications known to affect neural systems, had an intelligence quotient of ≤80, reported a history of serious head trauma, or had irremovable magnetically active objects on or within their body. To study the effects of MC phase on SC reactivity, additional exclusion criteria included males, pregnant or lactating females, post- or peri-menopausal females, females with irregular cycle length or outside of the range of 26–30 days, use of exogenous hormones and/or hormonal contraceptives and females currently experiencing any difficulties with their menstrual

cycle such as spotting between menses, severe painful menses, current diagnoses of premenstrual syndrome (PMS) or premenstrual dysphoric disorder (PMDD).

Thirty-seven physically healthy premenopausal females who exhibited chronic cigarette smoking behavior ranging in age from 20 to 51 years participated in the study (see Table 1). Eight individuals from a previous report who met the inclusion/exclusion criteria of the current study were included.[40] Following consent, participants completed psychological and physical evaluations. Participants received $100.00 for completing the fMRI scan and associated procedures. The study adhered to the Declaration of Helsinki and was approved by the University of Pennsylvania Institutional Review Board.

Measures

The Montreal International Neuropsychiatric Interview[41] was administered by a trained clinician and was used to assess current DSM-IV diagnosis of substance dependence and general psychiatric health. The Fagerstrom Test for Nicotine Dependence (FTND) assessed severity of nicotine dependence.[42,43] Craving was assessed by recording oral responses to the question "On a scale from 1 to 7 how much do you desire a cigarette right now?, with 1 corresponding to *Not at all* and 7 corresponding to *Extremely.*" The Menstrual Cycle Questionnaire (MCQ), developed within our laboratory, acquired information on MC characteristics and was used to identify MC phase. Information obtained from the MCQ included first day of last menses, MC length, regularity, and other characteristics of MC, including method of birth control. Based upon this information, females were either excluded or divided into luteal phase females (LPs; premenstrual) and follicular phase females (FPs; preovulatory) at time of scanning. The luteal phase was defined as the 14 days prior to the first day of menses and the follicular phase comprised the remaining days of the cycle.[44]

Imaging Approach

*p*CASL perfusion fMRI assessed brain activation in response to SC exposure. Prior to the scan session, participants smoked a cigarette to satiety in the presence of study personnel to minimize nicotine withdrawal-induced craving that might accrue during the scanning session and to standardize time since last cigarette. *p*CASL scans during cue exposure were acquired approximately 20–25 min after smoking to allow the acute cardiovascular effects of smoking to dissipate.

Ten-minute audio-visual clips were presented during *p*CASL scanning. The SC video included individuals differing in race, age, and sex who were smoking and using explicit language designed to induce appetitive desire for a cigarette ("I just can't wait to smoke this cigarette. It's been a couple of hours since I had one and this cigarette is going to taste so good."). The non-SC video was similar in content; however, the video did not portray cigarette smoking or smoking reminders. Different actors were used in the non-SC video to ensure that memory circuits would not be aroused. These actors also differed from each other in race, age, and sex and relayed interesting stories or anecdotes while brandishing a pen or pair of glasses or similar non-arousing object. The non-SC video was shown before the SC video to minimize interference in "carryover" arousal initiated when drug cues are shown first.[45,46] Craving was assessed prior to and immediately following SC exposure.

Imaging Data Acquisition

Imaging data were acquired on a 3.0 Tesla Trio whole-body scanner (Siemens AG). Data acquisition parameters were optimized over the course of the study. For co-registration of the functional data, a T1-weighted three-dimensional high resolution MPRAGE scan was acquired with field of view (FOV) = 160 mm, repetition time (TR) = 1,510 ms, echo time (TE) = 3 ms, 192 × 256 matrix, slice thickness 1 mm for 11 subjects (5 FP) and FOV = 250 mm, TR/TE = 1,620/3 ms, 192 × 256 matrix, slice thickness 1 mm for the remaining 26 subjects (17 FP). *p*CASL perfusion fMRI sequence was used to acquire SC and non-SC data. Interleaved images with and without labeling were obtained using a gradient echo echo-planar imaging sequence with a delay of 1,000 ms for 11 subjects or 700 ms for 26 subjects inserted between the end of the labeling pulse and image acquisition (FOV = 130 mm, matrix = 64 × 64 × 14, TR/TE = 3,000/17 ms, flip angle = 90°, slice thickness = 6 mm with a 2 mm inter-slice gap for 11 subjects and a 1.2 mm inter-slice gap for 26 subjects. To explore whether data acquisition differences affected findings, a homogeneity of variance test was applied across groups and no significant differences were observed.

Imaging Data Processing and Statistical Analyses

Data were preprocessed and analyzed using statistical mapping software, version 8 (SPM8; Wellcome Department of Cognitive Neurology; Http://www.fil.ion.ucl.ac.uk/spm/) implemented in MatlabR2013 (MathWorksInc.). An SPM-based arterial spin

Table 1. Participant Characteristics

	All	FPs	LPs	
	N = 37	*n* = 22	*n* = 15	*p*
Race (%)				.49
White	15 (41)	8 (36)	7 (47)	
Black	18 (49)	12 (55)	6 (40)	
Other	4 (10)	2 (9)	2 (13)	
Means ± (SEMs)				
Age	32.8 (1.6)	32.7 (2.1)	32.9 (2.6)	.94
Education	14.5 (0.3)	14.2 (0.4)	14.9 (0.5)	.31
Cigarettes per day	14.8 (1.2)	13.8 (1.4)	16.3 (1.9)	.30
Pack years[a]	11.9 (2.1)	11.7 (2.8)	12.2 (3.4)	.92
FTND scores[b]	4.6 (0.3)	4.4 (0.4)	4.8 (0.4)	.50

[a] Pack years calculation: Cigarettes per day (÷) cigarettes in a pack (X) years smoking.

[b] FTND = Fagerstrom Test for Nicotine Dependence; FTND scores ranged from 1.4 to 7.9.

labeling (ASL) data processing toolbox[47] was used for *p*CASL perfusion data analyses, as described previously.[34,48] Briefly, ASL image pairs were realigned to the mean of all control images and spatially smoothed with a 3D isotropic Gaussian kernel at 10 mm full width at half maximum (FWHM). For both SC and non-SC stimuli, 100 CBF image series were generated from the 100 label/control ASL image pairs using a simplified two-compartment model with the sinc interpolation method for CBF calculations.[49] The mean control image of each subject's data was co-registered to the structural image using the mutual information based co-registration algorithm provided by SPM8. The same transformation parameters were applied to co-register the CBF maps to each subject's anatomical image. Subsequently, the structural image was spatially normalized to the Montreal Neurological Institute (MNI) standard brain. The resulting transformation matrix was used to align the CBF images to MNI space. A gray matter brain mask was used to exclude non-gray matter areas in the CBF maps.

Contrasts between cue sets were defined in the general linear model to assess the voxel by voxel CBF difference for each subject. Using the corresponding parametric maps of the contrast, random effects analysis was employed to test for a significant main effect of condition (SC versus non-SC) in each group with a statistical parametric map of the *T*-statistic at each voxel for population inference within the gray matter mask. Given the ranges of the number of cigarettes smoked per day (CPD) and FTND scores were wide, these variables were entered in the model as covariates. Significant voxels passed a voxel-wise statistical threshold ($p < .005$) and, to control for multiple comparisons, were required to be part of a cluster >141 contiguous voxels, as determined by a Monte-Carlo simulation and resulted in 5% probability (corrected) of a cluster surviving due to chance. Associations between brain responses and SC-elicited craving (pre-SC vs. post-SC) were examined using linear regression analyses for each group. Pearson's correlation values were extracted from the clusters that passed multiple comparisons tests using the MarsBaR toolbox (http://marsbar.sourceforge.net/).

Demographic and Behavioral Statistical Analyses

Continuous demographic variables were summarized, by calculating means and standard error measurements (X ± SEMs). Independent samples *t* tests compared FPs and LPs on continuous variables. Chi-square analyses were used to compare nominal demographic variables. A repeated-measures analysis of variance (ANOVA) was used to assess the effect of group (FP vs. LP), time (pre-SC vs. post-SC), and the group X time interaction on SC-induced craving. Additional post hoc analyses were conducted to examine specific group differences. Analyses were conducted in Excel version 2008 (Microsoft) and SPSS version IBM SPSS Statistics 19.0.

Results

Subject Characteristics

Table 1 provides the means, SEMs, and *p* values for subject characteristics. The average MC length was 29.1 ± 0.9 days. One subject (FP) was left-handed. No significant differences were observed between FP and LP groups in general subject characteristics.

Smoking Cue-Induced Craving (Subjective)

Craving measured immediately before SC exposure (baseline craving) was 2.7 ± 0.3 in All subjects (FPs + LPs) with no differences observed between FPs (2.8 ± 0.4) and LPs (2.7 ± 0.4; $p = .6$). There was a significant main effect of exposure to SCs (pre-SC vs. post-SC) on craving ($F_{1,35} = 6.07$; $p = .02$). There was no group X time interaction ($F_{1,35} = 2.82$; $p = .10$). In post hoc analyses, we observed

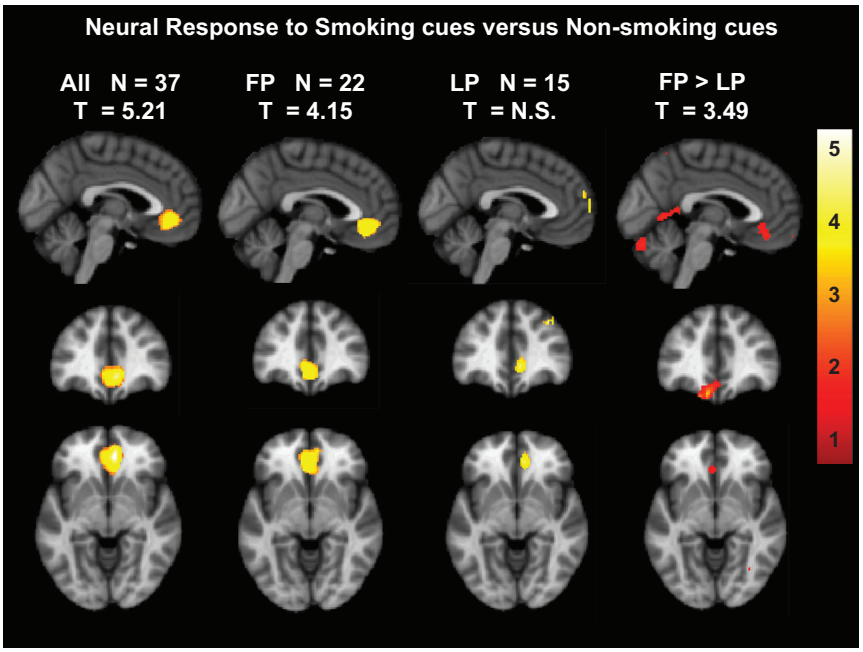

Figure 1. Representative fMRI sagittal, coronal, and axial brain images analyzed in SPM8 and overlain on the MNI brain showing neural responses to smoking cues relative to non-smoking cues in the medial orbitofrontal cortex in All subjects (FPs + LPs), follicular phase females (FPs), luteal phase females (LPs), and FPs versus LPs. Data are shown in MNI space at coordinates $x = -2$, $y = 38$, $z = -6$, which corresponds to the peak activation in the FPs. *T* Bar provides approximate *T* values. Images are displayed neurologically (left is left) at $p \leq .005$, $k = 141$ for All, FP and LP and $p \leq .005$, $k = 20$ for FP > LP.

significant increases in SC-elicited craving within FPs (4.2 ± 0.4; t_{21}= −3.1; p = .005) but not within LPs (3.0 ± 0.5; t_{14}= −0.6; p = .6).

Imaging Results

SC Versus Non-SC Reactivity

As shown in Figure 1, All subjects exhibited significantly greater neural responses to SCs relative to non-SCs in the mOFC (809 voxels; peak voxel: x, y, z = 6, 42, −6; T = 5.21). FPs showed similar responses in the same region (609 voxels; peak voxel: −2, 38, −6; T = 4.15). There were no *a priori* regions showing greater responses to SCs relative to non-SC cues in LPs, however there was an area of greater activation that extended ventrally from the middle frontal gyrus to the anterior cingulate (629 voxels; peak voxel 38, 34, 38; T = 6.53). FPs showed greater responses in the mOFC compared to LPs at the same threshold ($p \leq .005$) but the cluster size was less than that required for cluster correction (31 voxels). No other *a priori* regions in All subjects, FPs or LPs showed differences in SC relative to non-SC responses. A hypothesis-generating table (Supplementary Table 1) is available in supplementary material that lists non *a priori* regions for All subjects, FPs and LPs. There were no differences in any regions (*a priori* or non *a priori*) that met cluster correction in FPs versus LPs. Although CPD and FTND scores were used as covariates in the analyses, results were not significantly different when analyses were conducted without covariates.

In an attempt to remove interference in signal that may arise in females experiencing menstrual discomfort (early follicular) and potential ambiguity associated with phase determination (peri-ovulatory), post hoc analyses were conducted excluding females who were scanned on days 1–3 (N = 3) or days 13–17 (N = 2) of their cycle. Results were unchanged.

Correlations Between Brain Responses to SCs and Subjective SC-Induced Craving

In All subjects a correlation between SC-elicited craving and brain activity was observed within a large cluster (1,142 voxels) with its peak voxel of activation located within the inferior frontal gyrus (BA 44; −48, 14, 18; T = 4.39; r = 0.48; p = .002), spreading into the post central gyrus (BA 43; peak voxel: −54, −4, 16; T = 3.49) and the anterior insula (BA 13; peak voxel: −44, 10, 12; T = 3.90). As shown in Figure 2, FPs also showed a correlation in a large similarly located cluster (1,069 voxels) that extended from the inferior frontal gyrus (BA 9; peak voxel: −44, 8, 22; T = 4.51; r = 0.73; p < .0001) into the lentiform nucleus (peak voxel: −28, 14, −6; T = 3.69) anterior insula (BA 13; peak voxel: −38, 8, 8; T = 3.69) and ventral striatum (peak voxel: −26, 10, −8; T = 3.19). There were no correlations with SC-induced craving within *a priori* regions in LPs, which is likely related to the absence of SC-induced craving and truncated range of scores in this group. A hypothesis-generating table (Supplementary Table 2) is available in supplementary material that lists correlative relationships within non *a priori* regions for All subjects, FPs and LPs.

Discussion

The current study investigated the influence of MC phase on responses to SCs using an objective, neurobiological approach combined with subjective measures of SC-elicited craving. SC-elicited craving was reported by FPs but not by LPs. We replicated our previously published study in which we examined sex differences in SC reactivity,[40] by demonstrating that All subjects (FPs + LPs) exhibited significantly greater neural responses to SCs relative to non-SCs in the mOFC. When females were separated by MC phase, FPs showed similar responses in the same region while brain responses to SCs

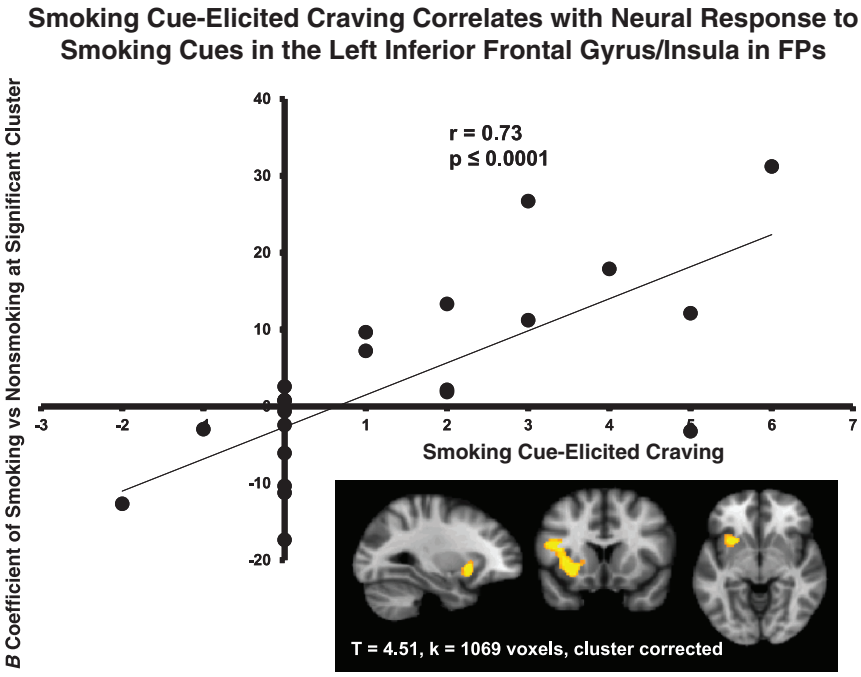

Figure 2. Graphical and pictorial representations of the correlation between smoking cue-elicited craving (*X* axis) and neural responses to smoking cues relative to nonsmoking cues (*Y* axis) in FPs. Representative fMRI sagittal, coronal, and axial brain images were analyzed in SPM8 and overlain on the MNI brain. Coordinates of the slice shown are −28, 14, −6. Images are displayed neurologically (left is left) at $p \leq .005$, k = 141. There were no correlations with smoking cue-elicited craving in *a priori* regions in LPs, which is likely related to the absence of SC-induced craving and truncated range of scores in this group.

relative to non-SCs were not significantly greater in *a priori* regions in LPs. In All subjects subjective reports of SC-elicited craving were positively associated with neural responses in a region spanning the inferior frontal gyrus, the post central gyrus and the anterior insula. FPs also showed an association between SC-elicited craving scores and neural responses in a similarly located region that included the inferior frontal gyrus and extended into the lentiform nucleus, the anterior insula and the ventral striatum, while correlations were not observed in LPs. These results provide initial evidence that females in the follicular phase of their MC have greater brain and behavioral responses to appetitive SCs compared to females in the luteal phase of their MC.

In our previously published study examining sex differences in SC reactivity females exhibited significantly greater neural responses in the mOFC. Here, in a larger cohort, we replicate that finding, however, we show that this effect may be at least in part, related to the influence of MC phase as the effect was significant only in FPs. The mOFC is involved in integrating endogenous (originating with the body) and exogenous (environmental) sensory information to guide behavior (decision making) for emotional rewards.[50,51] It is interesting to note, that activation to SCs is greater in FPs, suggesting that the SCs are rewarding, even while they have recently smoked to satiety. From an evolutionary perspective, experiencing greater reward from general and or specific stimuli during the follicular phase of the cycle, when a woman is physically able to conceive, has significance for survival in that she may be more available and receptive compared to when she is in the luteal phase and unable to conceive. Although strictly conjectural, experiencing greater reward from general or specific stimuli might also encourage risk-taking and discourage behavioral inhibition. Likewise, one might speculate that experiencing greater reward from smoking cigarettes (i.e., during a lapse) could convey greater vulnerability to relapse in females during this phase. In support of the interpretation that FPs experience the SCs as more rewarding, Dreher et al.[52] showed that women in the follicular phase of their MC, compared to when they are in the luteal phase, activated reward neurocircuitry during a monetary reward paradigm.[52]

This study showed that SC-elicited craving reports were associated with brain activity in the left hemisphere, specifically the left inferior gyrus, spreading into the post central gyrus and the anterior insula, and that this effect was most pronounced in FPs. Although both hemispheres of the brain play roles in emotional processing, controversy exists regarding which hemisphere is in control of which aspects of emotion. One hypothesis, referred to as the *Valence Specific Hypothesis* posits that the left hemisphere processes positive emotions while the right hemisphere processes negative emotions.[53] This hypothesis has been challenged by multiple studies in recent years including by Killgore and Yurgelun-Todd[54] who have shown that both hemispheres contributed to processing emotional stimuli irrespective of valence.[54] Nevertheless, it is interesting that repeatedly, including in the present study, associations between the left insula and craving have been observed in the SC literature[33,35,55,56] c.f.[57] Of note, Janes et al.[58] conducted an fMRI study specifically designed to examine how SCs are cognitively processed. They used a delay match to sample task during brain scan acquisition, which differentiates between the encoding, maintenance, and retrieval phases of working memory. They found that a large cluster that spanned left inferior gyrus, claustrum and the anterior insula was activated specifically during the retrieval (recognition) of previously viewed smoking-related images.[58] Note, that this cluster corresponds to the same area of the brain that correlates with SC-elicited craving in the current study.

In a previous study we examined the influence of MC phase on self-reported SC-induced cigarette craving.[32] In conflict with the current findings, FPs reported less SC-induced craving compared to LPs. One important difference between the previous and current studies is that different smoking-related stimuli were used. In Franklin et al[32] the smoking stimulus was a 15-min video portraying the activities of a heterosexual White American male on a day he is scheduled for a job interview. He smokes cigarettes throughout the video including while dressing for the interview, while having coffee with his wife prior to leaving and while waiting for the bus. Preparing for, and participating in a job interview is a highly stressful activity that may arouse negative affect. In contrast, the SCs used in the current study consist of a 10-min video that was specifically designed to be highly appetitive, and cigarettes are depicted as exceedingly rewarding; individuals happily relate anecdotes about how much they enjoy smoking while puffing on their cigarette. The difference is in the emotional impact that each cue set may have on the participants. The earlier set of SCs may evoke feelings of stress and/or anxiety, that individuals may perceive could be relieved by smoking, while the SCs used in the current study may evoke feelings of pleasantness that individuals may perceive could be enhanced by smoking. Thus, although not directly tested here, it might be posited that across the two studies SC-induced craving was elicited to a greater extent in LPs when a negative mood was triggered and to a greater extent in FPs when a positive mood was triggered. The greater response in the mOFC in FPs during SC exposure supports this inference, as this region is more active during the presentation of positive stimuli.[59]

Limitations

The results of this initial study should be interpreted cautiously. Importantly, MC phase was not biochemically verified and thus MC phase was used as a proxy for ovarian hormone function. Although others have demonstrated that paradigmatic measures of verification align with self report of MC phase,[60,61] knowledge of MC phase does not necessarily translate to the hormonal milieu; over one third of MCs are anovulatory and hormones do not fluctuate normally during anovulatory cycles.[62] Thus, even if MC phase was correctly identified for the subjects in the current study, one must be cautious in extrapolating knowledge of MC phase to knowledge of hormonal status. Additionally, females were not randomized prospectively by MC phase and thus selection bias is a potential caveat. Indeed, we acknowledge that the most scientifically rigorous test of our hypothesis would be to scan females during SC exposure at both phases of the MC (biochemically verified). Additionally, it would be important to study postmenopausal females who have decreased levels of gonadal hormones. Ongoing SC reactivity studies in our laboratory are currently acquiring biochemical confirmation of gonadal hormone levels in pre- and postmenopausal females to conduct a more rigorous examination.

Conclusions

The results presented here suggest that MC phase modulates responses to appetitive reminders to smoke cigarettes. FPs display greater brain responses in the reward-related mOFC and increased craving during exposure to appetitive SCs compared to LPs. These results should be considered in the context of the significant health problem of chronic cigarette smoking and its effects on females. Females face increased smoking-related deleterious health consequences compared to males,

including increased risk of lung cancer and reproductive health problems.[1] These factors emphasize the importance of identifying sex-specific relapse predictors and revealing underlying neurobiological mechanisms that will aid in the development of effective smoking cessation strategies. This initial foray into understanding the role of hormones in SC reactivity warrants the attention of researchers, and underscores the importance of monitoring menstrual cycle phase/hormonal status in all areas of addiction research.

Supplementary Material

Supplementary Tables 1 and 2 can be found online at http://www.ntr.oxfordjournals.org.

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Declaration of Interests

None declared.

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