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Subjective, Physiological, and Cognitive Responses to Intravenous Nicotine: Effects of Sex and Menstrual Cycle Phase

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Nicotine dependence is a serious public health concern. Optimal treatment of nicotine dependence will require greater understanding of the mechanisms that contribute to the maintenance of smoking behaviors. A growing literature indicates sex and menstrual phase differences in responses to nicotine. The aim of this study was to assess sex and menstrual phase influences on a broad range of measures of nicotine response including subjective drug effects, cognition, physiological responses, and symptoms of withdrawal, craving, and affect. Using a well-established intravenous nicotine paradigm and biochemical confirmation of overnight abstinence and menstrual cycle phase, analyses were performed to compare sex (age 18–50 years; 115 male and 45 female) and menstrual cycle phase (29 follicular and 16 luteal) effects. Females had diminished subjective drug effects of, but greater physiological responses to, nicotine administration. Luteal-phase females showed diminished subjective drug effects and better cognition relative to follicular-phase women. These findings offer candidate mechanisms through which the luteal phase, wherein progesterone is dominant relative to estradiol, may be protective against vulnerability to smoking.

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INTRODUCTION

Cigarette smoking remains the main preventable cause of death in developed countries (Danaei *et al*, 2009). Fewer women than men smoke and female smokers consume fewer cigarettes, yet the gender gap in population smoking rates is narrowing and women smokers may face greater health risks than men (eg, lung cancer) (USDHHS, 2001). Women may be less likely to achieve long-term abstinence than men (see, eg, Cepeda-Benito *et al*, 2004, but also see Munafo *et al*, 2004). If the ratios of 'former' to 'ever' smokers reached equivalence between the sexes, it would translate to ~1 million additional women quitting (Perkins, 2009b).

Smoking is reinforced by alleviation of aversive withdrawal-associated symptoms, pursuit of rewarding aspects (Allen *et al*, 2008b, 2009), and improvement on aspects of cognitive functioning (Leventhal *et al*, 2007; Merritt *et al*, 2012). These factors affect smoking maintenance and relapse-risk (see, eg, Allen *et al*, 2008b, 2009). Sex differences in subjective, cognitive, and physiological nicotine responses may provide clinically relevant mechanistic explanations for sex differences in smoking maintenance and cessation patterns (for review, see Benowitz and Hatsukami, 1998).

Women and men may differentially experience nicotine abstinence or administration. Short-term abstinent women smokers reported greater withdrawal symptoms (al'Absi *et al*, 2002), negative affect, withdrawal-related distress, and smoking urges than men. Although some studies report no sex differences in abstinence-related decrements (Leventhal *et al*, 2007) or during *ad libitum* smoking, sex-sensitive abstinence-related decrements have been observed in certain cognitive measures (eg, divided but not selective attention; Merritt *et al*, 2012).

Studies examining nicotine's rewarding effects have reported sex differences, although findings are mixed. In a series of studies, Perkins (2009a) found women smokers less able to discriminate or self-titrate intranasal nicotine doses than men, suggesting women's smoking behavior is reinforced more by nonnicotine cues (for review, see Perkins, 2009a). Women report greater sensitivity to subjective effects of oral (Netter *et al*, 1994), intranasal (Myers *et al*, 2008), intravenous (Sofuoglu and Mooney, 2009), or transdermal (Evans *et al*, 2006) nicotine than men.

Mechanisms underlying these sex differences are unclear, yet changes in hormones across menstrual cycle phases may contribute. Estradiol and progesterone are neuroactive and interact with neurotransmitter systems implicated in addiction (Anker and Carroll, 2011; Lynch *et al*, 2002).

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Studies examining the role of menstrual cycle phase on outcomes related to nicotine addiction have yielded inconsistent findings. Although numerous studies have reported increased withdrawal severity during the luteal phase (for review, see Carpenter *et al*, 2006), others reported greater withdrawal or craving in the follicular phase (Allen *et al*, 2009), or no phase effects on withdrawal (see, eg, Masson and Gilbert, 1999; Pomerleau *et al*, 1992) or acute intranasal nicotine administration (Marks *et al*, 1999). These conflicting findings may arise from methodological differences. Many studies have not biochemically verified phase or distinguished between withdrawal and premenstrual symptoms (for review, see Carpenter *et al*, 2006).

Smoking behavior and cessation outcomes are linked with gonadal hormone levels. A recent study found that higher progesterone to estradiol ratios predict diminished laboratory smoking behavior (Schiller *et al*, 2012). Smoking cessation trials provide support for phase effects on smoking cessation. A large ($N=202$), well-controlled study (Allen *et al*, 2008a) found women who quit in the (biochemically determined) follicular phase, compared with those who quit during the progesterone-dominant luteal phase, relapsed to smoking faster. Similar findings were reported in women receiving bupropion (Mazure *et al*, 2011). Trials including nicotine replacement therapy (NRT) plus behavioral treatment found better outcomes in women quitting in the follicular phase (Carpenter *et al*, 2008; Franklin *et al*, 2008). These discrepant findings were hypothesized to arise from differential inclusion of NRT across trials (Franklin and Allen, 2009). These findings suggest that modulation of nicotine's effects by gonadal hormones and phase may affect smoking and quitting patterns.

The main purpose of this study was to investigate sex and menstrual phase contributions to subjective, physiological, and cognitive responses to intravenous nicotine in smokers following overnight abstinence. We hypothesized that women, relative to men, would have: (1) greater subjective effects of nicotine *vs* placebo; (2) greater withdrawal, craving, and negative affect; and (3) less cognitive impairment after overnight abstinence and less cognitive improvement after the session. We further hypothesized that women in luteal phase, relative to follicular phase, would show: (1) attenuated subjective nicotine effects; (2) greater withdrawal and craving symptoms following overnight abstinence and less alleviation of these symptoms by nicotine administration.

MATERIALS AND METHODS

Participants

Nontreatment-seeking cigarette smokers (115 male and 45 female; aged 18–50 years) were recruited from the New Haven, Connecticut area. Smoking status was defined as 10–25 cigarettes/day for the past year, Fagerström Test of Nicotine Dependence (FTND; Pomerleau *et al*, 1994) $\geqslant 5$, and expired carbon monoxide (CO) >10 parts per million (p.p.m.). Participants were medically healthy, did not meet criteria for Axis I psychiatric disorders, including dependence on alcohol or drugs other than nicotine, and were not using psychotropic medication or were not pregnant or breastfeeding.

Procedures

Screening session. An in-person screening session, held ~ 2 weeks before the experimental session, determined eligibility and collected written informed consent and background data. Participants were paid following participation. The VA Connecticut Healthcare System Human Subjects Subcommittee approved the study.

The Structured Clinical Interview for DSM-IV (SCID; First *et al*, 1996) was used to screen for Axis I psychiatric disorders. A physician health check and laboratory test battery ensured general medical health.

Experimental session. Participants were asked to abstain from smoking and food from midnight before the session at 0800 h. Participants continued their usual caffeine intake to avoid caffeine withdrawal symptoms. Drug and pregnancy urine screens were administered. An indwelling catheter with multiple ports in an antecubital vein collected blood samples and administered saline and nicotine.

Baseline biochemical measures were collected before saline or nicotine delivery. Serum progesterone and estradiol levels in women determined luteal ($\geqslant 2$ ng/ml progesterone) *vs* follicular (<2 ng/ml progesterone) phase. Expired CO ($\leqslant 8$ p.p.m.) and plasma nicotine concentrations verified overnight abstinence (Benowitz *et al*, 2002). Nicotine metabolites cotinine and 3′-hydroxycotinine (3HC) assessed past nicotine use (Benowitz *et al*, 2002) and contributed to the nicotine metabolite ratio (NMR; 3HC/cotinine). NMR, a relatively stable indicator of nicotine clearance rate, may vary by sex and respond to large fluctuations in gonadal hormone levels (eg, pregnancy), but is not modulated by phase (Benowitz *et al*, 2006; Hukkanen *et al*, 2005).

After baseline measures were collected, participants received IV saline, then two escalating weight-adjusted nicotine doses (0.5 mg/70 kg, 1.0 mg/70 kg), 30 min apart. These doses have previously been shown to be well tolerated, yet produce robust physiological and both positive and negative subjective effects in men and women (Sofuoglu and Mooney, 2009; Sofuoglu *et al*, 2008, 2009, 2011, 2012). Doses were administered in an escalating manner to avoid nicotine carryover into the saline dose and as a safety precaution. Injections were administered 30 min apart to provide sufficient time for subjective and physiological measures to approach baseline (Sofuoglu *et al*, 2008; Sofuoglu and Mooney, 2009).

Subjective nicotine effects, assessed with the Drug Effects Questionnaire (DEQ), were collected before (-5 min) and then 1, 3, 5, 8, and 10 min following each saline and nicotine delivery. The DEQ is a 10-item visual analog scale (100 mm converted to 1–10 rating).

Heart rate and systolic and diastolic blood pressure were collected before (-5 min) and 1, 2, 3, 5, 8, 10, and 15 min after saline and each nicotine delivery.

Plasma cortisol levels were collected at baseline, before each injection, and at the end of session. Plasma cortisol may differ by sex and show modest sensitivity to nicotine withdrawal (Pickworth and Fant, 1998) and administration (Mendelson *et al*, 2005, but also see al'Absi *et al*, 2002).

Measures of withdrawal (Minnesota Nicotine Withdrawal Scale (MNWS) (Hughes and Hatsukami, 1986), craving

(Brief Questionnaire of Smoking Urges (BQSU); Cox *et al*, 2001), and affect (Positive and Negative Affect Schedule (PANAS); Watson *et al*, 1988) were collected at baseline and end of session.

A computerized cognitive assessment, consisting of three tasks from the ANAM (Automated Neuropsychological Assessment Metrics) battery (Reeves *et al*, 2002) was completed at baseline and end of session. The selected tasks are described briefly below and in the Supplementary Materials.

The Stroop task consisted of three stimulus levels: (1) 'word': color words written in white ink, (2) 'color ': 'XXX' displayed in colored ink, and (3) 'incongruent': color words displayed in incongruent ink colors (eg, 'red' in blue ink). Participants pressed one of three colored buttons corresponding to the word ('word') or ink color ('color', 'incongruent'). 'Incongruent' performance taps response inhibition and cognitive control, as participants must override prepotent word-reading tendencies and respond to ink colors. Outcome variables were: 'interscore' (ie, correct responses during 'incongruent' *vs* 'word' and 'color' trials); 'level 3 throughput' (ie, 'incongruent' correct responses within the available time); and 'mean correct response time (RT)' within each level.

In the running memory continuous performance task (CPT), participants pressed one of two buttons to indicate whether a letter was the same as the previously presented letter. CPT assesses sustained attention and taps working memory.

In the mathematical processing task (MPT), participants pressed one of two buttons to indicate whether a 3-integer equation (eg, $4+6-3$) solution was greater or less than 5. MPT taps basic computational skills, attention, and working memory.

Outcome measures for CPT and MPT were: 'throughput,' 'mean correct RT,' and 'percent correct.' These tasks were chosen for the sensitivity of similar tasks to nicotine withdrawal and administration (Myers *et al*, 2008).

Data Analyses

Sex. Males ($N=115$) and females ($N=45$) were compared on demographic and baseline variables using analysis of variance (ANOVA) for continuous, or χ^2 for categorical, variables.

Repeated-measures models included within- and between-subject factors (mixed-models) in Statistical Analysis System, version 9.2 (SAS Institute, 2009).

Heart rate, blood pressure, and DEQ analyses included dose (saline, 0.5 mg/70 kg nicotine, 1.0 mg/70 kg nicotine), min after dose, sex, and dose-by-min, sex-by-dose, sex-by-min, and sex-by-dose-by-min contrasts. Cortisol analyses included dose (presaline, pre-0.5 mg/70 kg nicotine, pre-1.0 mg/70 kg nicotine, end of session), sex, and sex-by-dose contrasts. Cognitive, withdrawal, craving, and affect measure analyses included time point (baseline, end of session), sex, and sex-by-time point contrasts.

Values of $p\leqslant 0.05$ in two-tailed tests were considered statistically significant.

Menstrual cycle phase. Analyses outlined above were rerun, replacing the between-subject factor of sex with phase (follicular and luteal).

RESULTS

Results for baseline group differences are presented in Table 1; influences of sex and phase are presented in Table 2; other statistics are presented in Supplementary Tables S1–S5. One woman reported hormonal birth control (depo provera) use. She was not an outlier on any measures of interest (demographic, baseline clinical, outcome) and hence was not excluded.

Sex

There were no baseline sex differences except for higher BMI in women (Table 1).

As a manipulation check, DEQ was sensitive to nicotine administration, with most subscales showing higher ratings for nicotine than saline (Figure 1, Supplementary Figure S1, and Supplementary Tables S1 and S2).

Men rated DEQ items 'stimulated' and 'feel good' higher than women. Sex-by-dose interactions reflected increases in 'stimulated' and 'feel good' ratings in men with each dose, with women rating both nicotine doses equivalently, yet higher than saline. Men rated 0.5 mg/70 kg nicotine as more 'stimulating' than women but did not differ on other doses. Men rated 0.5 mg/70 kg as more 'feel good' than women rated 1.0 mg/70 kg nicotine (Table 2 and Figure 1). Despite no overall sex differences in 'anxious' ratings, sex-by-dose interaction reflected lower anxiety in men *vs* women at saline and diminishing anxiety ratings in women with each dose, with men rating lower 'anxiety' during 1.0 mg/70 kg nicotine dose compared with saline or 0.5 mg/70 kg nicotine dose.

Heart rate and systolic and diastolic blood pressure peaked 1 min after dose in a dose-dependent manner (Figure 1, Supplementary Figure S1, and Supplementary Tables S1 and S2). Men had lower heart rate overall and a sex-by-dose interaction reflected greater nicotine dose-related heart rate increases in women than men. Sex-by-dose interactions reflected higher systolic blood pressure in men than women with saline, increases in both sexes at both nicotine doses, but no significant sex differences at each nicotine dose. Similarly, sex-by-dose interactions for diastolic blood pressure reflected increases by dose with no significant sex differences at each dose (Table 2, Figure 1, and Supplementary Figure S1).

Subjects reported decreased withdrawal (MNWS), smoking urges (BQSU), and positive and negative affect (PANAS) at end of session *vs* baseline, but no statistically significant effects of sex or sex-by-time point (Table 2 and Supplementary Tables S1 and S3).

Subjects improved on cognitive measures at end-of-session *vs* baseline, showing more correct and faster responses across tasks, but no effects of sex or sex-by-time point (Table 2 and Supplementary Tables S1, S3).

Menstrual Cycle Phase

There were no baseline phase differences except for higher progesterone and plasma cotinine levels in the luteal than follicular phase (Table 1).

Within the women-only phase analysis sample, nicotine doses increased heart rate and blood pressure relative to

Table 1 Baseline Measures for the Study Sample by Sex and Phase

Measures	Sex analyses[a]			Menstrual cycle phase analyses[b]		
	Men (*N* = 115)	**Women (*N* = 45)**	**Statistics[c]**	**Follicular (*N* = 29)**	**Luteal (*N* = 16)**	**Statistics[c]**
Demographics	***N* (%)**	***N* (%)**	***X*²(*p*)**	***N* (%)**	***N* (%)**	***X*²(*p*)**
Race			·			·
African American	47 (40.9)	26 (57.8)	·	58.6	56.3	
Caucasian	58 (50.5)	14 (31.1)		31.0	31.3	
Native American	1 (0.9)	1 (2.2)		3.4	0	
Biracial/Other	9 (7.8)	4 (8.9)	·	6.9	12.5	
Hispanic ethnicity	20 (17.4)	4 (8.9)		10.3	6.3	·
	Mean (SD)	**Mean (SD)**	**F(p)**	**Mean (SD)**	**Mean (SD)**	**F(p)**
Age, years	37.0 (8.9)	37.5 (8.3)	·	38.9 (7.9)	35.1 (8.6)	·
Body mass index (BMI)[d]	28.0 (4.4)	31.9 (7.7)	4.0 (<0.001)	32.9 (7.7)	30.1 (7.7)	·
Weight (lbs)[e]	191.9 (32.4)	190.0 (51.1)		197.6 (52.1)	176.3 (47.6)	·
Self-reported smoking history and severity						
Fagerström Test of Nicotine Dependence (FTND)	5.4 (2.1)	31.9 (7.7)	·	6.1 (2.1)	5.5 (1.8)	·
Average cigarette consumption/day	18.7 (13.0)	19.3 (10.3)	·	20.4 (10.3)	17.3 (10.4)	·
Age onset regular smoking	17.0 (5.1)	15.6 (3.0)	·	15.7 (3.1)	15.4 (2.7)	·
Estimated years of smoking[f]	20.0 (9.4)	21.9 (8.2)	·	23.1 (7.8)	19.7 (8.8)	·
Biochemical smoking indices at baseline						
Plasma cotinine, ng/ml	198.8 (132.8)	214.11 (146.46)	·	196.5 (149.1)	246.0 (140.4)	4.2 (0.046)
Plasma 3'-hydroxycotinine (3HC)	67.0 (47.2)	76.7 (51.4)	·	73.7 (50.8)	82.0 (53.6)	·
Nicotine metabolite ratio (NMR; 3HC/cotinine)[g]	0.37 (0.19)	0.42 (0.27)	·	0.48 (0.31)	0.33 (0.16)	·
Plasma nicotine, ng/ml[h]	3.18 (3.68)	2.8 (2.2)	·	2.3 (1.9)	3.7 (2.6)	·
Gonadal hormone levels at baseline[i]						
Progesterone, ng/ml[j]	n/a	4.1 (6.5)	n/a	0.76 (0.41)	10.0 (7.9)	40.2 (<0.001)
Estradiol, ng/ml	n/a	123.6 (126.9)	n/a	133.4 (153.6)	105.9 (52.1)	·

No other significant menstrual cycle phase differences were observed on demographic, smoking history or severity, baseline biochemical smoking indices, or estradiol levels.

[a]Baseline measures for sample included in sex difference analyses.

[b]Baseline measures for sample included in phase difference analyses.

[c]Statistics (F(*p*) or $\chi^2(p)$ as appropriate) are reported for results that reached statistical significance at $p<0.05$ level. '·' Indicates nonsignificant group differences.

[d]The female sample had higher average BMI than the male sample. No other demographic or baseline smoking history or severity measures significantly differed by sex.

[e]Intravenous nicotine doses were weight-adjusted (0.5 or 1.0 mg/70 kg). Despite sex differences in BMI, sex or phase groups did not significantly differ by weight.

[f]Estimated years of smoking was derived from age at testing and age of onset of regular smoking and does not account for periods of nicotine abstinence.

[g]Nicotine metabolite ratio data could not be computed for 11 subjects (9 males and 2 females).

[h]Plasma nicotine levels were not available for one male subject.

[i]Estradiol and progesterone levels were only collected in women, and are therefore not available (n/a) in men.

[j]Progesterone levels were significantly higher in the luteal phase relative to the follicular phase as expected as these levels were used to determine phase.

saline, but no statistically significant effects of phase or phase-by-dose were observed (Table 2, Supplementary Tables S1 and S4, and Supplementary Figure S1).

Women reported nicotine effects on DEQ ratings (except 'anxious' and 'feel down'), with higher ratings for both nicotine doses than saline, but doses were rated equivalently (Table 2, Supplementary Tables S1, S4 and Figure S1, Figure 1).

Ratings of 'high' were lower in the luteal than follicular phase and dose-by-phase interactions reflected greater dose-related changes in the follicular than luteal phase on ratings of 'high,' 'want more,' 'feel good,' and 'sedated.' The follicular-phase group reported increased 'high' and 'want more' for both doses (*vs* saline), whereas the luteal-phase group reported increases for 0.5 mg/70 kg nicotine (*vs* saline) but rated 'high' and 'want more' from 1.0 mg/70 kg

Table 2 Effects of Sex and Phase on Responses to Nicotine Doses and Experimental Session

Measure	Sex analyses[a]		Menstrual cycle phase analyses[b]	
A. Sex and phase differences by dose	**Sex**	**Sex by dose[c]**	**Phase**	**Phase by dose[c]**
Subjective	*F*(p)	*F*(p)	*F*(p)	*F*(p)
Drug Effects Questionnaire				
High	·	·	L<F; 5.82 (0.019)	7.76 (<0.001)
Stimulated	W<M; 5.00 (0.027)	3.42 (0.033)	·	·
Feel good	W<M; 4.94 (0.027)	·	·	3.67 (0.026)
Like	·	·	·	·
Want more	·	·	·	4.99 (0.007)
Drug strength	·	·	·	·
Sedated	·	·	*L<F; 3.20 (0.080)*	3.42 (0.033)
Anxious	·	5.26 (0.005)	·	·
Feel bad	·	*2.63 (0.072)*	·	·
Feel down	·	*2.45 (0.086)*	·	·
Physiological and biochemical				
Vital statistics				
Heart rate	M<W; 12.38 (<0.001)	12.61 (<0.0001)		·
Diastolic blood Pressure	·	3.84 (0.022)		*2.37 (0.094)*
Systolic blood Pressure	·	9.45 (<0.0001)		·
Cortisol[d]				
Plasma cortisol	W<M; 13.82 (<0.001)			·
B. Sex and phase differences by time point	**Sex**	**Sex by time point[e]**	**Phase**	**Phase by time point[e]**
Subjective	*F*(p)	*F*(p)	*F*(p)	*F*(p)
Minnesota Nicotine Withdrawal Scale				
Total MNWS score	*M<W; 3.85 (0.052)*	·	·	·
Brief Questionnaire Smoking Urges				
BQSU factor 1	·	·	·	*2.98 (0.092)*
BQSU factor 2	·	·	·	·
Positive and negative affect schedule				
PANAS positive affect	·	·	·	·
PANAS negative affect	·	·	L<F; 5.22 (0.028)	·
Cognitive Task Performance[f]				
Stroop task				
Throughput: 'Incongruent' (L3)	·	·	*F<L; 3.27 (0.079)*	·
Mean correct RT: 'Incongruent' (L3)	·	·	*L<F; 3.68 (0.063)*	·
Continuous Performance Task (CPT)				
Throughput	·	·	*F<L; 3.39 (0.074)*	·
Percent correct	·	·	·	·
Mean correct RT	·	·	L<F; 4.85 (0.034)	·

Abbreviations: W, women; M, men; F, follicular phase; L, luteal phase; RT, response time.
Results in italics reached trend levels of significance (>0.05, <0.1). '·' Indicates results that did not reach statistical significance or trend significance levels.
[a]Results from sex difference analyses (115 men and 45 women). Effects of dose, min, dose by min, sex by min, and sex by dose by minute are reported in Supplementary Table S1.
[b]Results from phase difference analyses (29 follicular and 16 luteal). Effects of dose, min, dose by min, phase by min, and phase by dose by min are reported in Supplementary Table S2.
[c]Three dose levels were included in the analyses: placebo (saline), 0.5 mg/70 kg nicotine, and 1.0 mg/70 kg nicotine.
[d]Cortisol was collected at four time points: before each dose (saline, 0.5 and 1.0 mg/70 kg nicotine) and at end of session.
[e]Cognitive measures (Stroop, CPT, and MPT) and measures of withdrawal (MNWS), craving (BQSU), and affect (PANAS) were collected at two time points only; at the beginning (baseline) and end of the experimental session.
[f]There were no significant or trend effects of sex, sex by time point, phase, or phase by time point on the primary Mathematical Processing Task output measures (throughput, percent correct, mean correct RT) or for Stroop task Interference Score 'Incongruent' condition (level 3), mean correct RT for 'word' (level 1) or 'color' (level 2), or the difference in mean correct RT at level 3 relative to levels 1 and 2 combined.

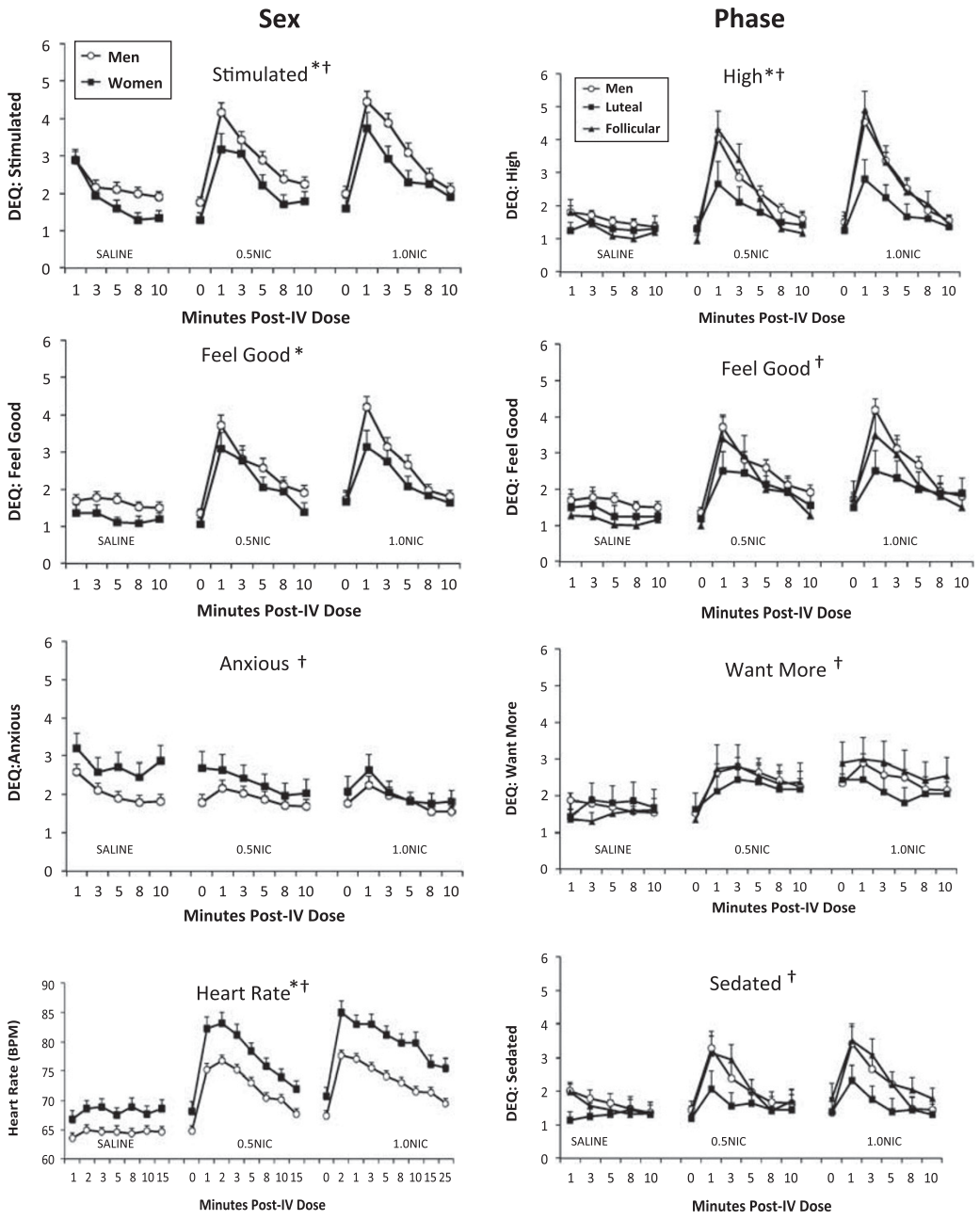

Figure 1 Influence of sex and phase on subjective and physiological IV dose effects. *Significant ($p \leqslant 0.05$) effect of sex or phase; †significant ($p \leqslant 0.05$) sex-by-dose or phase-by-dose interaction.

as equivalent to saline. The follicular-phase group reported increases in 'feel good' ratings with each dose, whereas the luteal group reported equivalent increases with both nicotine doses. 'Sedation' ratings increased with nicotine (*vs* saline) in the follicular but not luteal phase (Table 2, Supplementary Tables S1 and S5, and Figure 1).

Overall, withdrawal, craving, and affect scores decreased at the end of session *vs* baseline (Table 1 and Supplementary Tables S1 and S5). The follicular-phase group reported higher negative affect (PANAS) than the luteal group. A trend-level phase-by-time point interaction in craving (BQSU Factor 1) reflected decreased craving at the end of session (*vs* baseline) in the follicular but not the luteal group (Table 2, Supplementary Tables S1 and S5, and Figure 2).

Women improved performance across cognitive measures (except MPT percent correct) at end of session *vs* baseline. The luteal-phase group performed better than the follicular group on cognitive measures, including faster correct CPT responses. No phase-by-dose interactions were observed (Table 2, Supplementary Tables S1 and S5, and Figure 2).

DISCUSSION

Despite the absence of sex differences on baseline smoking severity and biochemical nicotine indicators, men reported greater subjective reactivity to nicotine, whereas women

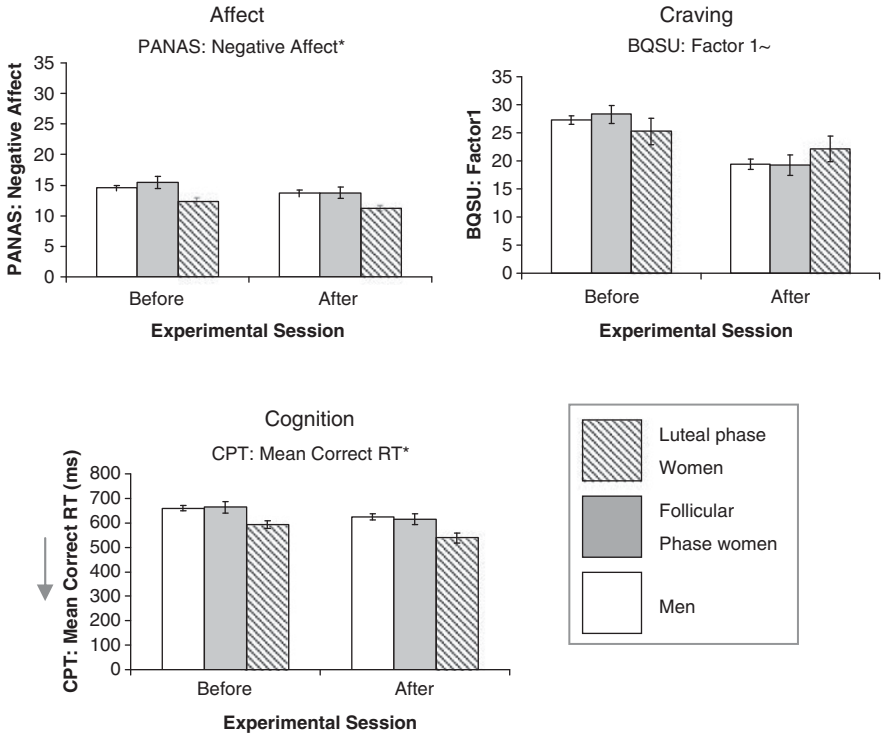

Figure 2 Influence of menstrual cycle phase on affect, cognitive performance, and craving. *Significant ($p \leqslant 0.05$) effect of phase; ~trend ($0.5 < p < 0.1$) effect of phase-by-timepoint; ↓indicates that lower scores represent more optimal cognitive task performance.

showed greater physiological reactivity to nicotine. Women in the luteal, relative to follicular, phase reported diminished subjective nicotine reactivity, fewer negative affect symptoms, and better cognitive task performance. These findings add to a growing literature showing sex differences in acute nicotine responses, and provide new evidence for the influence of the menstrual cycle phase on these outcomes.

Women reported diminished subjective experiences of predominately positively valenced nicotine effects relative to men. This finding was contrary to our hypothesis and inconsistent with our previous findings from a smaller sample using the same IV infusion paradigm (Sofuoglu and Mooney, 2009), and with studies of oral (Netter *et al*, 1994), intranasal (Myers *et al*, 2008), and transdermal (Evans *et al*, 2006) nicotine administration. As phase was not previously accounted for (Sofuoglu and Mooney, 2009), and our findings implicate phase in nicotine's subjective effects, differential proportions of women in each phase could feasibly have contributed to conflicting sex difference findings, although this cannot be confirmed with existing data. Our findings of greater subjective nicotine sensitivity in men were consistent with previous reports with intranasal nicotine (Perkins, 2009a) and smoked cocaine (Sofuoglu *et al*, 1999). The lack of main effects of sex on more negatively valenced DEQ subscales and presence of dose-sensitive results suggests these findings were likely not attributable to global sex differences in response to intravenous delivery or in reporting subjective effects. That women's smoking behaviors may be more driven by smoking-related cues than men's smoking behaviors (Perkins, 2009a) was unlikely to explain our results, as many smoking-related cues were deliberately minimized by the IV nicotine paradigm. Furthermore, sex differences in cue reactivity would be expected to influence positively and negatively valenced subjective nicotine effects similarly. However, as hypothesized, women reported less subjective distinction between nicotine dose levels, consistent with diminished nicotine dose discrimination sensitivity in women (for review, see Perkins, 2009a).

Our findings did not support previous reports of women having greater craving and negative affect than men (al'Absi *et al*, 2002; Leventhal *et al*, 2007), although we found a nonsignificant trend toward higher withdrawal in women than men. We did not observe sex differences for cognitive performance. However, the impact of phase on these measures (see, eg, O'Hara *et al*, 1989) suggests that differences in the relative proportions of women in each phase could contribute to variation in sex difference findings across studies. Previous findings of sex-sensitive effects of nicotine on cognition varied across cognitive domains and difficulty levels, and thus were not generalized effects (see, eg, Merritt *et al*, 2012).

The findings of greater physiological responses to nicotine in women than men are consistent with previous findings of greater heart rate responses to high dose transdermal nicotine (Evans *et al*, 2006) and greater diastolic blood pressure during *ad libitum* smoking in women than men (Merritt *et al*, 2012), yet inconsistent with studies finding no sex differences in heart rate or blood pressure responses to nicotine (see, eg, (Leventhal *et al*, 2007; Sofuoglu and Mooney, 2009)). Future research could assess

whether greater physiological reactivity to nicotine among women contributes to tendencies for women to suffer more adverse smoking-related health consequences than men (USDHHS, 2001).

Consistent with our hypotheses, women in the luteal (*vs* follicular) phase reported diminished subjective nicotine effects. Phase differences were more prominent at 1 mg/70 kg nicotine. Women's attenuated ratings and diminished dose discrimination compared with men may have been accounted for by diminished ratings at the higher doses among women in the luteal phase. Our findings are consistent with previous reports of attenuated subjective effects of cocaine (Sofuoglu *et al*, 1999) and amphetamine (Justice and de Wit, 1999) in luteal *vs* follicular phases. Given raised progesterone levels in the luteal phase, our findings are also consistent with reports of exogenous progesterone diminishing subjective effects of cigarettes (Sofuoglu *et al*, 2001, 2011), intravenous nicotine (Sofuoglu *et al*, 2009), and cocaine (Sofuoglu *et al*, 2004).

Women in the follicular, relative to luteal, phase reported greater negative affect symptoms. Women in the follicular phase also reported decreased urges to smoke in pursuit of rewarding symptoms at the end of the experimental session, whereas women in the luteal phase did not. These measures were not assessed following each dose, and hence may not have been entirely attributable to nicotine, rather than generalized experimental session or time effects. However, taken together with our other findings, cravings may be more effectively satisfied following nicotine administration for women in the follicular *vs* luteal phase, because the latter experienced diminished subjective nicotine effects. These findings may shed light on the conflicting results in the literature wherein studies of short-term nicotine abstinence tend to show higher withdrawal or craving measures in the follicular phase (or low progesterone conditions; see, eg, Allen *et al*, 2009; Sofuoglu *et al*, 2001, 2009, 2011) whereas *ad libitum* smoking studies tend to show higher levels in the luteal phase (see, eg, DeBon *et al*, 1995; Pomerleau *et al*, 1992).

Women in the luteal, compared with follicular, phase tended to perform better across measures of cognitive control (Stroop) and sustained attention (CPT). However, phase did not differentially influence nicotine administration (or repeat-testing) effects on performance. These results are consistent with our previous finding that progesterone improved overnight abstinent smokers' Stroop and psychomotor speed performance, but progesterone effects were not differentially modulated by nicotine (Sofuoglu *et al*, 2011).

Our findings of no significant phase effects on physiological measures were consistent with those from a previous study in naturally cycling women (Marks *et al*, 1999), although other studies found effects of exogenous hormones (ie, oral contraception (Masson and Gilbert, 1999) and progesterone (Sofuoglu *et al*, 2011)) on physiological responses to nicotine. Therefore, greater variations in hormone levels may affect physiological measures more than phase.

Strengths and Limitations

The intravenous nicotine paradigm offers strengths and limitations relative to cigarette smoking paradigms. Intravenous nicotine allows for more precise dosing and timing of delivery because of individual differences in smoking topography (eg, puff volume). Intravenous nicotine is well-tolerated by males and females (see, eg, Sofuoglu and Mooney, 2009) and preferred by smokers to placebo (Sofuoglu *et al*, 2008). Although this paradigm allows for a more accurate assessment of nicotine's (cigarettes' primary addictive component) pharmacological effects, it does not assess other tobacco components or smoking cue-related processes that are important in maintaining smoking behaviors and may be modulated by sex or phase (see, eg, Perkins, 2009a).

Another strength of this study was the biochemical verification of overnight abstinence and assessment of NMR, a factor relevant to subjective nicotine response (Sofuoglu *et al*, 2012) and previously proposed to contribute to sex differences in nicotine response (Benowitz *et al*, 2006). Importantly, sex and phase groups did not differ on NMR in this sample, suggesting our findings were likely not accounted for by nicotine metabolism differences. Furthermore, sex and phase groups did not differ in smoking history or severity, and hence the results were unlikely to be artifacts of clinical severity differences.

Inclusion of several different domains of nicotine effects was a strength; however, the testing approach did have limitations. Subjective and physiological effects were assessed at multiple time points after saline and nicotine administrations, allowing for assessment of dose- and time-related effects. Cognitive, withdrawal, craving, and affect measures were assessed such that it is not possible to parse out the effects of nicotine from the saline, time-of-day, or test–retest effects for these measures. For example, significant cognitive improvements across time points may indicate persistent nicotine-induced cognitive amelioration, greater task familiarity at retest, or higher afternoon alertness. Craving may fluctuate with time of the day (Allen *et al*, 2009), but this would not explain observed differential changes in craving by phase. Numerous measures were administered multiple times, and hence effects of test–retest or fatigue could have contributed to findings.

Limitations related to the sample are noteworthy. First, this study recruited nontreatment-seeking smokers, primarily because of ethical considerations surrounding administering nicotine to treatment seekers. Although inclusion of nontreatment-seeking smokers offers more representative indicators of mechanisms of smoking maintenance as treatment seekers may have ambiguous attitudes towards nicotine, the observed findings may not apply to treatment-seeking individuals. Second, despite a substantial overall sample size, an unequal sex ratio arose from more study-eligible men responding to advertisements than women. The resulting modest sample size for phase analyses may have diminished statistical sensitivity and prohibited comparison of men with each phase subgroup. Third, the sample of smokers was otherwise physically and mentally healthy, and therefore may not be representative of naturalistic samples of smokers. This approach was taken as a safety precaution given the intravenous nicotine paradigm (eg, excluded for hypertension), and to diminish variance related to comorbid psychiatric conditions. Finally, women were not screened for perimenopausal symptoms.

Between-subject phase analyses, perhaps resulting in greater variance and lower statistical sensitivity, allowed for data

acquisition in a single experimental session avoiding complications related to expectations of IV nicotine effects.

Our findings have several implications. First, menstrual cycle phase effects may contribute to sex differences in nicotine's actions, suggesting phase should be accounted for in studies of sex differences in nicotine abstinence or administration. Second, our findings provide mechanisms by which luteal phase may facilitate smoking cessation, including less severe negative affect and cognitive decrement following overnight abstinence and attenuated subjective nicotine responses, outcomes shown to contribute to smoking relapse (see, eg, Allen *et al*, 2008b). Our findings suggest the luteal phase diminishes nicotine's positive and negative reinforcing properties. Third, these observed phase effects support the role of sex hormones in tobacco addiction and may provide a mechanistic explanation for findings of diminished laboratory smoking behavior when endogenous progesterone to estradiol ratios are higher (Schiller *et al*, 2012), such as is the case during the luteal phase. Our findings provide additional support for progesterone as a potential smoking cessation aid in women.

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AIH, AJW, and GWV declare no conflict of interest. EED was partially reimbursed by the College on Problems of Drug Dependence (CPDD) and NIDA for costs of attending CPDD Annual Meeting 2013 to present these data as part of a symposium and has no other conflict of interest to declare. MS serves as an expert witness on behalf of Pfizer in lawsuits related to varenicline. The funding bodies had no role in the collection, analysis, or the decision to publish these data.

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