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Early onset cigarette smokers exhibit greater P300 reactivity to smoking-related stimuli and report greater craving

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Abstract

Adolescence is a period during which a number of critical neuromaturation processes occur and the vulnerability for developing nicotine dependence is extremely high. Thus, early-onset (EO; age < 16 years old), relative to late-onset (LO; age ≥ 16 years old), tobacco smoking may be uniquely deleterious for developmentally immature systems that regulate neural signaling reactivity. This study investigated how age of tobacco smoking onset affects neurophysiological measures of smoking cue reactivity and reported craving in adult smokers. EO smokers (EOS; n = 8; 4 females), LO smokers (LOS; n = 10; 5 females), and healthy non-smokers (HNS; n=10; 5 females) participated in an event-related potential (ERP) cue reactivity study with tactile and image stimuli. Participants handled neutral objects during one interval and smoking-related objects during a second interval. After each interval, they viewed smoking-related, neutral, or arousing images using an oddball paradigm. P300 ERPs and craving for tobacco were recorded during each session. P300 amplitudes were significantly higher in central midline (Cz) channel to smoking, but not neutral or arousing, images after handling smoking objects. Specifically, Cz P300 smoking amplitudes were significantly greater in EOS, relative to LOS and HNS, and associated with greater craving at baseline. There were no other group differences in mood or craving. EOS exhibited greater P300 reactivity to smoking-related stimuli, relative to LOS, suggesting a more sensitized neural response. EO smoking during early neuromaturation may alter

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Contributors

YM and SEL designed the study. JB and SLF oversaw participant recruitment, and assisted with data collection and organization. YM conducted the data analysis and wrote the manuscript. SEL, JB, and SLF made contributions and edited the manuscript. YM consolidated edits from coauthors. All authors approved the final manuscript.

Conflicts of interest

Authors do not have any conflicts of interest to disclose.

Compliance with ethical standards

All procedures were approved by the McLean Hospital Institutional Review Board and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

neurophysiological signaling involved in responding to smoking-related stimuli, which could impact the outcome of smoking cessation interventions.

Keywords

smoking; early onset; P300; event-related potentials; cue reactivity; EEG

1. Introduction

Tobacco smoking among adolescents remains a persistent threat to public health (Ng et al., 2014). Nationwide population data shows that, among adolescents aged 12 to 17, approximately 850,000 are recently initiated cigarette smokers and approximately 1.2 million recently reported current cigarette smoking (SAMHSA, 2015). Previous reports have shown that nearly 80% of adult chronic smokers began smoking prior to age 18 (CDC, 2014), and commensurate with these data, individuals who initiate smoking during adolescence are more likely to have greater difficulty quitting as an adult and to become lifetime smokers (Taioli and Wynder 1991; Sussman, 2002).

Adolescence represents a vulnerable period for smoking initiation and dependence that also temporally coincides with crucial brain maturation processes. Cortical synaptic pruning and white matter myelination refinements persist throughout adolescence and early adulthood, and plateau at approximately ages 24–25 (Gogtay et al 2004; Tamnes et al., 2010). These developmentally critical processes are particularly important for greater neural signaling efficiency, normative neurotransmitter release, and strengthening of neural connections (Rakic et al., 1994; Dwyer et al., 2009). The lasting effects of nicotine on the adult brain are determined in part by the neurodevelopmental stage, such as early or late adolescence, at which nicotine exposure is initiated (Trauth et al. 1999). More specifically, the neurobiological impact of early-onset smoking, defined as smoking initiated prior to age 16, may be especially deleterious because maturational changes in active synaptic pruning and rearrangement (Huttenlocher 1979, 1990) and white matter myelination (Paus et al., 1999) begin to accelerate during early adolescence. Age 16 also has been used consistently in substance abuse research as a dividing mark for early- vs. late-onset drug use (Ehrenreich et al., 1999; Slade et al., 2008; Norberg et al., 2009; Becker et al., 2010; Gruber et al., 2012; Sagar et al., 2015; Dahlgren et al., 2016).

Persistent exposure to nicotine via tobacco smoking throughout adolescent neurodevelopment can damage newly maturing synaptic signaling pathways and alter patterns of neurotransmitter release, increasing sensitivity to reward-related neural activation and susceptibility to developing nicotine dependence (Abreu-Villaça et al., 2003a; Dwyer et al., 2009). To this end, preclinical findings have shown distinct differences in nicotine self-administration behaviors between adult rats treated with nicotine during either periadolescence or postadolescence (Adriani et al. 2003). Periadolescent rats treated with nicotine between postnatal days 34–43 (an age range comparable to ‘early onset’ or early adolescence (Arrant et al. 2013)), compared to postadolescent rats treated with nicotine between postnatal days 60–69 (an age range comparable to ‘late onset’ or late adolescence/

early adulthood), exhibited greater motivation and higher rates of responding to self-administer nicotine when tested as adults (approximately postnatal day 105) (Adriani et al. 2003). Thus, periadolescent (e.g. 'early onset') exposure to nicotine can produce distinct sensitized responses to the salient appetitive properties of the drug that correspond to greater self-administration behavior and facilitate the transition to long-term drug dependence in adulthood (Morgan et al. 2006).

Investigating the effects of early or late onset adolescent smoking on maintenance of smoking behaviors in adulthood, which also could affect and interfere with smoking cessation attempts, will help define the pathophysiology of nicotine dependence. Elevated reactivity to smoking-related cues (i.e. 'smoking cue reactivity'), such as smoking-related images or cigarette smoke, is hypothesized to play a critical role in the maintenance of nicotine dependence and in increasing relapse vulnerability if a quit attempt is made (Niaura et al., 1988; Rohsenow et al., 1990; Janes et al., 2010). Exposure to smoking-related objects, (e.g., a cigarette box, cigarettes, or an ashtray), relative to neutral objects (e.g. a pen or pad of paper similarly sized to a cigarette box), is also an important determinant of smoking craving and reactivity (LaRowe et al., 2007). Additionally, behavioral measures of anxiety, withdrawal, depressive feelings, impulsivity, and craving reported by smokers are also known to influence cigarette smoking behaviors and cue reactivity (e.g. Doran et al., 2009; Weinberger et al., 2012). Overall, examining if early onset adult smokers exhibit greater smoking cue reactivity to smoking-related images and objects, relative to late onset adult smokers, who initiated smoking at or after 16 years old, will give some indication of functional differences between the two groups in smoking cue sensitivity. Furthermore, investigating relationships between elevated cue reactivity and mood and craving measures will reveal if different aspects of subjective affect or craving reported by EOS and LOS specifically influence smoking cue reactivity.

One approach for modeling smoking cue reactivity in the laboratory is through the use of electroencephalography (EEG) and event-related potentials (ERPs). EEG directly measures continuous neuronal electrical activity within the cortical surface of the brain. A methodological strength of EEG is millisecond-range high temporal resolution, which facilitates synchronization of cortical electrical activity with specific experimental events, such as responding elicited by repeated image stimulus presentations (Hajcak et al., 2010). The EEG signal encompasses positive and negative voltage fluctuations over time that, when time-locked to a discrete event or defined stimulus, are called ERPs (Hajcak et al., 2010; Wallois et al., 2012). ERPs are commonly defined by the polarity and approximate latency of the voltage deflection in the EEG signal. Thus, the 'P300' ERP is a positive waveform that peaks approximately 250–550 milliseconds following stimulus presentation (Kemper et al., 2012). Notably, P300 amplitude increases following presentation of an infrequent target or task-relevant stimulus, suggesting an increase in the degree of cortical information processing and attentional resource allocation that is taking place (Kok 1990; Polich and Criado 2006). Other factors that influence P300 amplitude and latency include attentional resource redirection and incentive saliency of novel stimuli (Spencer et al., 2001; Polich and Criado 2006; Mantini et al., 2009). The P300 reflects automatic motivational attention processing and allocation to salient stimuli (Kujawa et al., 2013) whereas a longer latency ERP, the late positive potential (LPP), is considered to be sensitive to sustained elaborative

processing of emotionally salient stimuli (Weinberg and Hajcak, 2011). Our *a priori* intent is to measure automatic reactivity related to the motivational salience and relevance of smoking-related cues, which are properties known to support drug-seeking and drug-taking behaviors (Robinson and Berridge, 1993), thus our focus is on the P300 component.

Drug cue reactivity oddball paradigms are commonly used to study the associations between brain electrical reactions and drug-related image stimuli presentations. Three-stimulus oddball paradigms, in particular, utilize a sequence of stimuli presentations in which rare infrequent ‘target’ stimuli and rare infrequent non-target stimuli are randomly inserted in a series of frequently presented neutral stimuli (Katayama and Polich, 1998). Studies indicate that drug-dependent patients will redirect attentional resources to highly salient target or distractor drug-related images, which increases corresponding P300 amplitudes (Littel et al., 2012; Robinson et al., 2016). Relative to frequent neutral picture cues and healthy non-drug-using adults, P300 amplitudes in drug-dependent adults increased in response to target heroin- (Lubman et al., 2007), cocaine- (Penetar et al., 2012), smoking- (Littel and Franken, 2011; Evans et al., 2013), and marijuana-related (Nickerson et al., 2011; Henry et al., 2014) picture cues. Furthermore, each of these three-stimulus oddball P300 studies included target non-drug picture cues, which did not elicit significant P300 reactivity comparable to the specificity of elevated P300 drug cue reactivity. Less is known, however, about P300 smoking cue reactivity measured by a three-stimulus oddball paradigm during acute nicotine deprivation. Changes in P300 amplitude and latency to smoking-related cues have not been characterized in early and late onset adult smokers but could elucidate novel patterns of neural reactivity that are elicited by the incentive value of tactile and visual cues and influenced by nicotine exposure during early or late stages of brain maturation.

Previous smoking cue reactivity studies have revealed that P300 amplitudes measured from midline Fz, Cz and Pz locations in cigarette smokers were larger in response to smoking-related than neutral cues (Warren and McDonough 1999; McDonough and Warren 2001; Littel and Franken 2007; Littel and Franken 2011; Luijten et al., 2016). Centro-parietal channels typically reflect a strong P300 response elicited by rare target stimuli (Brown et al., 2015), therefore the primary focus of the present study was on Cz and Pz reactivity. Given the strong centro-parietal topography of the P300 response, Fz P300 amplitudes were assessed as a secondary analysis. Findings of Fz reactivity to smoking-related imagery are sometimes inconsistent, with some observing higher P300 amplitudes (e.g., Warren and McDonough 1999; Littel and Franken 2007) and others observing less pronounced reactivity (Engelmann et al., 2011). P300 ERP were used to explore whether early and late onset smokers exhibit differences in smoking cue reactivity and if reactivity was altered by handling neutral or smoking-related objects. It was hypothesized that early onset smokers would exhibit greater P300 amplitudes in Cz and Pz electrode sites in response to viewing smoking-related images after handling smoking-related objects and would further report greater craving prior to and during the EEG P300 session compared to late onset smokers and healthy non-smokers. It was also hypothesized that measures in LOS would be greater than those in HNS. Additional analyses of associations between significant changes in P300 amplitudes, craving and mood will offer insights into functional consequences of early onset smoking.

2. Results

2.1. Demographics and substance use assessments

All participant demographic and substance use variables (means $\pm$ SD) are reported in Table 1. Participants were well-matched, with no significant differences in age and education. Smokers did not exhibit differences in FTND scores. Given the focus of age of smoking onset in the current study, significant differences between smokers in age at regular cigarette smoking and in duration of smoking exposure were expected. Length of smoking history is an important factor in assessing functional differences between EOS and LOS, thus duration of smoking exposure, in years, was used as a covariate in all ANCOVA analyses.

As expected, EOS and LOS exhibited greater CO levels at study baseline than HNS, but the two groups did not differ from each other. LOS also reported significantly more alcohol use per week than EOS and HNS.

2.2. Baseline mood, smoking withdrawal, and craving assessments

All baseline mood variables (means $\pm$ SD) are reported in Table 2, and all baseline withdrawal and craving variables (means $\pm$ SD) are reported in Table 3. Some group differences emerged in smokers in baseline mood measures. Specifically, relative to HNS, LOS reported higher BDI scores and higher POMS Anger and Confusion subscale scores. Additionally, relative to HNS, EOS exhibited higher scores on the BIS Motor subscale. Furthermore, both EOS and LOS, relative to HNS, reported higher scores on the BIS Total scale, POMS Tension subscale, and STAI State Anxiety inventory.

There were no differences between smokers in their scores on the WSWS but EOS did exhibit higher craving on the QSU-Brief, reporting a higher score than LOS on “Nothing would be better than smoking a cigarette right now” and a higher Total score.

2.3. Primary outcomes: between-condition

2.3.1. Cz P300 amplitudes and peak latency—RM ANCOVAs, controlling for sex and duration of smoking exposure, revealed a significant main effect of condition, indicating that midline Cz P300 amplitudes were significantly greater in response to smoking-related cues after handling smoking-related objects (Smoking condition) ($F(1,23) = 4.4$, $p = 0.05$, $ES=0.43$) (Figure 1).

There was also a significant interaction of condition $\times$ group, ($F(2,23) = 11.95$, $p = 0.00$, $ES=0.72$). Table 4 lists Cz P300 amplitudes (means $\pm$ SD) exhibited by EOS, LOS, and HNS to stimulus images (neutral, arousing, and smoking-related) in each condition as a reference for making direct comparisons. *Post hoc* group comparisons revealed that, in the Smoking condition, Cz P300 smoking cue reactivity was significantly greater in EOS, relative to LOS ($p = 0.01$) and HNS ($p = 0.01$) (Figure 2). Comparisons also showed that Cz P300 amplitudes were lower in LOS relative to HNS ($p = 0.02$) (Figure 2), indicating that LOS Cz P300 reactivity in the Smoking condition was lower in contrast to the Neutral condition. Importantly, there were no statistically significant interactions for condition $\times$ duration of smoking exposure ($F(1,23) = 3.6$, n.s.) or for condition $\times$ sex ($F(1,23) = 3.8$, n.s.).

We did note that there was a group difference in number of alcohol drinks reported per week. To determine if recreational alcohol use would significantly modulate reactivity to smoking cues, RM ANCOVAs, controlling for sex, duration of smoking exposure, and number of drinks per week, were consequently performed on Cz P300 smoking cue reactivity between sessions. There was a significant main effect of condition ($F(1,22) = 4.18$, $p = 0.05$, $ES=0.44$) as well as a significant interaction of condition x group ($F(2,22) = 8.06$, $p = 0.002$, $ES=0.64$). *Post hoc* group comparisons, consistent with our *a priori* analysis, also revealed that, in the Smoking condition, Cz P300 amplitudes were significantly greater in EOS, relative to HNS ($p = 0.001$) and to LOS ($p = 0.002$). The analyses further indicated that Cz P300 amplitudes were lower in LOS relative to HNS ($p = 0.03$). Importantly, there was no significant interaction for condition x drinks per week ($F(1,22) = 0.03$, n.s.), or for condition x sex ($F(1,22) = 3.7$, n.s.) or condition x duration of smoking exposure ($F(1,22) = 3.4$, n.s.). Overall, analyses indicate that alcohol use in participants likely did not influence cue reactivity between conditions.

No significant main effects were observed for Cz P300 amplitudes for neutral ($F(1,23) = 0.00$, n.s.) or arousing ($F(1,23) = 0.04$, n.s.) image stimuli between Neutral and Smoking conditions. Thus, handling smoking-related objects, particularly by EOS and LOS, did not increase non-specific reactivity to arousing image stimuli.

There were no differences in Cz peak latency between Neutral and Smoking conditions for neutral ($F(1,23) = 2.35$, n.s.), arousing ($F(1,23) = 0.02$, n.s.), or smoking-related ($F(1,23) = 0.10$, n.s.) image stimuli.

2.3.2. Pz P300 amplitudes and peak latency—RM ANCOVAs revealed a trend for the main effect of condition for parietal midline (Pz) P300 amplitudes in response to smoking cues in the Smoking condition ($F(1,23)=3.85$, $p=0.06$) but pairwise comparisons did not reveal any significant group differences in Pz smoking cue reactivity. Pz amplitudes did not differ between Neutral and Smoking conditions for neutral ($F(1,23) = 1.62$, n.s.) or arousing ($F(1,23) = 0.42$, n.s.) image stimuli.

There were no significant differences in Pz peak latency between conditions for neutral ($F(1,23) = 0.29$, n.s.), arousing ($F(1,23) = 0.02$, n.s.), or smoking-related ($F(1,23) = 2.9$, n.s.) image stimuli.

2.3.3 Fz P300 amplitudes and peak latency—No significant main effects were observed in the frontal midline (Fz) channel between Neutral and Smoking conditions for either neutral ($F(1,23) = 0.78$, n.s.), arousing ($F(1,23) = 0.20$, n.s.), or smoking-related ($F(1,23) = 0.16$, n.s.) image stimuli.

There were no significant differences in Fz peak latency between conditions for neutral ($F(1,23) = 1.0$, n.s.), arousing ($F(1,23) = 0.20$, n.s.), or smoking-related ($F(1,23) = 0.1$, n.s.) image stimuli.

2.4. Craving and mood assessments across EEG session

RM ANCOVAs, controlling for sex and duration of smoking exposure, analyzed mood and craving reported during 4 time points assessed across the study session and revealed there were no group differences related to changes in mood or craving at any time point. Participants did not report feeling anxious ($F(3,69) = 2.14$, n.s.), upset ($F(3,69) = 2.6$, n.s.), or happy ($F(3,79) = 1.5$, n.s.) as a result of neutral object handling, viewing the first picture presentation block, smoking-related object handling, or viewing the second picture presentation block. Similarly, EOS and LOS did not differ in their self-reports of craving across the same 4 time points when asked “How strong is your desire to smoke cigarettes,” ($F(3,42) = 0.29$, n.s.; mean response EOS: 91.3 ± 7.0 ; mean response LOS: 92.5 ± 3.7), “How strong is your desire to smoke right now?” ($F(3,42) = 0.29$, n.s.; mean response EOS: 90.7 ± 11.5 ; mean response LOS: 92.3 ± 2.2), and “How strong is your urge to smoke when your environment reminds you of it?” ($F(3,42) = 0.85$, n.s.; mean response EOS: 91.3 ± 2.0 ; mean response LOS: 87.1 ± 2.3).

2.5. Primary outcomes: within-condition

2.5.1. Cz P300 amplitude and peak latency—One-way ANCOVA analyses of group differences of smoking cue reactivity in the Neutral condition revealed a significant main effect of group indicating that after handling neutral objects, elevated Cz P300 amplitudes were driven by group differences in reactivity to smoking-related images ($F(4,23) = 3.9$, $p = 0.02$, $ES=0.81$). Pairwise comparisons indicated that both EOS ($p = 0.002$) and LOS ($p = 0.001$) exhibited greater reactivity to smoking-related images than HNS. There were no group differences in Cz P300 reactivity in the Neutral condition for neutral ($F(4,23) = 0.6$, n.s.) or arousing ($F(4,23) = 2.46$, n.s.) image stimuli. There were no significant differences in Cz peak latency for neutral ($F(4,23) = 0.7$, n.s.), arousing ($F(4,23) = 1.3$, n.s.), or smoking-related ($F(4,23) = 1.7$, n.s.) image stimuli.

Within-condition ANCOVA analyses of cue reactivity in the Smoking condition similarly demonstrated a significant main effect of group indicating that, after handling smoking-related objects, elevated Cz P300 amplitudes were also driven by group differences in reactivity to smoking-related images ($F(4,23) = 7.0$, $p = 0.001$, $ES=1.1$). All significant pairwise comparisons are already described above (Figure 2). There were no group differences in Cz P300 reactivity in the Smoking condition for neutral ($F(4,23) = 1.5$, n.s.) or arousing ($F(4,23) = 1.3$, n.s.) image stimuli. There were no significant differences in Cz peak latency for neutral ($F(4,23) = 0.2$, n.s.), arousing ($F(4,23) = 0.7$, n.s.), or smoking-related ($F(4,23) = 2.3$, n.s.) image stimuli.

2.5.2. Pz P300 amplitude and peak latency—There were no group differences in Pz P300 reactivity in the Neutral condition for neutral ($F(4,23) = 0.2$, n.s.), arousing ($F(4,23) = 0.7$, n.s.), or smoking-related ($F(4,23) = 2.0$, n.s.) image stimuli. Furthermore, no significant differences were observed in Pz peak latency for neutral ($F(4,23) = 0.3$, n.s.), arousing ($F(4,23) = 1.2$, n.s.), or smoking-related ($F(4,23) = 1.5$, n.s.) image stimuli.

Similarly, there were no group differences in Pz P300 reactivity in the Smoking condition for neutral ($F(4,23) = 0.5$, n.s.), arousing ($F(4,23) = 0.3$, n.s.), or smoking-related ($F(4,23) =$

0.4, n.s.) image stimuli. Furthermore, no differences were observed in Pz peak latency for neutral ($F(4,23) = 0.6$, n.s.), arousing ($F(4,23) = 0.9$, n.s.), or smoking-related ($F(4,23) = 1.9$, n.s.) image stimuli.

2.5.3. Fz P300 amplitude and peak latency—One-way ANCOVA analyses did not find any group differences in Fz P300 reactivity in the Neutral condition for neutral ($F(4,23) = 0.9$, n.s.), arousing ($F(4,23) = 2.0$, n.s.), or smoking-related ($F(4,23) = 1.5$, n.s.) image stimuli. Furthermore, no significant differences were observed in Fz peak latency for neutral ($F(4,23) = 0.5$, n.s.), arousing ($F(4,23) = 0.5$, n.s.), or smoking-related ($F(4,23) = 1.5$, n.s.) image stimuli.

There were no group differences in Fz P300 reactivity in the Smoking condition for neutral ($F(4,23) = 1.7$, n.s.), arousing ($F(4,23) = 1.6$, n.s.), or smoking-related ($F(4,23) = 1.4$, n.s.) image stimuli. No differences were observed in Fz peak latency for neutral ($F(4,23) = 1.0$, n.s.), arousing ($F(4,23) = 1.7$, n.s.), or smoking-related ($F(4,23) = 0.9$, n.s.) image stimuli.

2.6. Correlations: Cz P300 reactivity and clinical measures

Significant positive correlations were reported between Cz P300 smoking cue reactivity after handling smoking-related objects and baseline QSU-Brief craving, specifically “Nothing would be better than smoking a cigarette right now” ($r=0.50$, $p = 0.04$) (Figure 3). No other significant associations were observed between Cz P300 smoking cue reactivity and any group differences in clinical, withdrawal, or mood measures reported either at baseline or during the session.

2.7. Secondary outcomes

The three groups did not differ on any secondary outcomes, including stimulus response RT, response accuracy, and errors of commission and omission for each set of stimulus images measured between Neutral and Smoking conditions. RM ANCOVAs indicated that when responding to neutral images, groups did not differ in response RT ($F(1,23) = 0.31$, n.s.), accuracy ($F(1,23) = 2.0$, n.s.), or errors of commission ($F(1,23) = 0.06$, n.s.) and omission ($F(1,23) = 1.5$, n.s.). When responding to arousing non-drug-related images, groups did not differ in response RT ($F(1,23) = 1.6$, n.s.), accuracy ($F(1,23) = 0.1$, n.s.), or errors of commission ($F(1,23) = 0.65$, n.s.) and omission ($F(1,23) = 0.03$, n.s.). When responding to smoking-related images, groups did not differ in response RT ($F(1,23) = 0.03$, n.s.), accuracy ($F(1,23) = 3.1$, n.s.), or errors of commission ($F(1,23) = 1.2$, n.s.) and omission ($F(1,23) = 2.3$, n.s.).

3. Discussion

EOS exhibited higher cortical reactivity to smoking-related images after handling smoking-related cues, and also reported greater levels of baseline cigarette craving, relative to LOS. Overall, the data revealed elevated Cz P300 reactivity in EOS, relative to LOS and HNS, after viewing salient smoking-related images during the Smoking condition relative to the Neutral condition. Though there were no group differences in baseline WSWS withdrawal scores, EOS reported greater baseline levels of cigarette craving than LOS, as measured by

the QSU-Brief, indicating “Nothing would be better than smoking a cigarette right now.” Both groups reported high levels of craving throughout the EEG session but there were no significant differences between EOS and LOS in subjective craving levels. Elevated Cz P300 smoking cue reactivity was positively correlated, however, with baseline levels of cigarette craving, which was an effect primarily driven by the difference between EOS and LOS. Thus, while craving may not have fluctuated significantly between groups during the course of exposure to smoking-related objects and images, acute craving experienced by EOS, relative to LOS, may have persisted throughout the session and contributed to elevated smoking cue reactivity.

The findings from the present study align with previous studies showing elevated P300 reactivity and processing bias to smoking-related cues, relative to neutral cues, in smokers (Warren and McDonough, 1999; McDonough and Warren, 2001; Littel and Franken, 2007; Littel and Franken 2011; Bloom et al., 2013), but extend these findings to indicate that the incentive-motivational value of smoking-related stimuli and reactivity towards smoking-related cues was greater in EOS relative to LOS. Additionally, our findings suggest that elevated smoking cue reactivity in EOS occurred independently of smoking history, given that our analyses accounted for group differences in smoking history by controlling for duration of smoking exposure. The elevated P300 smoking cue reactivity exhibited by adult EOS suggests that young smokers undergoing early maturational stages may be uniquely vulnerable to the *strength* of associative reinforcing relationships formed between cigarette smoking and smoking-related objects and/or imagery. These data, in admittedly small but well-characterized cohorts, are the first to suggest distinct profiles of smoking cue reactivity in adult smokers grouped by early or late age of cigarette smoking onset.

The divergent finding of elevated smoking cue reactivity in EOS, relative to LOS, raises the intriguing possibility that EOS are at a distinct risk for strengthening dysfunctional associative reward pathways that promote neural reactivity to reinforcing smoking-related stimuli. Early adolescence is characterized by hyper-responsive incentive reward neurocircuitry and reward processing, and immature prefrontal executive control circuitry, that is less pronounced in late adolescence (Van Leijenhorst et al., 2010; Gogtay et al., 2004). As such, early onset smokers may be more vulnerable to biobehavioral reactivity to rewarding and reinforcing stimuli that may precipitate behavioral sensitization (e.g. enhanced responsivity to drug stimuli (Kalivas and Stewart 1991)). Preclinical models of periadolescent (comparable to ‘early onset’ or early adolescence (Arrant et al., 2013)) nicotine exposure also indicate evidence of an elevated biobehavioral vulnerability and increased sensitivity to the reinforcing and motivational properties of nicotine (Adriani et al., 2003). Rats pretreated in periadolescence with nicotine, relative to rats pretreated in postadolescence (comparable to ‘late onset’ or late adolescence/early adulthood), exhibit higher nicotine self-administration behaviors in adulthood, suggesting that early-onset nicotine exposure results in greater prolonged sensitization to the reinforcing efficacy of nicotine (Adriani et al., 2003). Postadolescent nicotine pre-treatment did not produce an enhanced sensitized response to nicotine self-administration in adult rats, suggesting that late-onset exposure affects the motivational properties of nicotine and can produce less reactivity to external nicotine-related stimuli (Adriani et al., 2003). Relationships formed between smoking and smoking-related stimuli may not take hold on reward association

pathways as rigidly in late adolescence, thereby decreasing the magnitude of a sensitized response to smoking-related stimuli in LOS. Thus, while LOS did react in the Neutral condition to smoking-related imagery after handling neutral objects, this group failed to exhibit a similar magnitude of significantly heightened reactivity to smoking-related imagery after handling salient smoking-related objects as EOS. More specifically, the low reactivity observed in LOS in the Smoking condition, in contrast to the reactivity observed in EOS, likely reflects the lack of a sensitized response. In fact, comparisons indicated that HNS exhibited greater reactivity to smoking-related imagery in the Smoking condition than LOS, which was likely driven by the situational novelty for HNS of handling smoking-related objects and consequent processing of updated stimulus representation when viewing smoking-related images (Polich, 2007). Increased reactivity of cigarette smokers to smoking stimuli plays an important role in smoking relapse susceptibility (Niaura et al., 1988; Rohsenow et al., 1990; Janes et al., 2010). Elevated cortical reactivity in EOS to smoking-related stimuli, compared to LOS, suggests that EOS may be more vulnerable to smoking relapse if a quit attempt is made (Donohue et al., 2016).

Cortical activity that is proximal to the Cz scalp electrode is influenced by sensory and motor functions through sensorimotor cortex processing, which is activated by the complexity and concentrated attention of a visuomotor task (Teplan 2002; Milnik et al., 2013). Furthermore, the sensorimotor cortex has previously exhibited enhanced cerebral metabolic activity that was strongly associated with handling cigarettes and increased subjective craving in chronic smokers (Brody et al., 2002). Presently, it is possible that increased Cz neural electrical activity measured in EOS was strongly influenced, and possibly modulated, by elevated craving and handling smoking-related objects (including cigarettes) prior to a salient visuomotor cue reactivity task. Furthermore, each of the visual cue stimuli were linked to a specific required motor response (to press one button for all smoking-related images and a different button for all neutral and arousing images) that also may have influenced P300 activity. The probability of a target smoking-related image presentation (20%), however, was the same as the probability of an arousing non-drug-related image presentation (20%), thus balancing the different button responses for equally 'rare' image presentations. Our findings of elevated Cz P300 smoking cue reactivity in EOS are consistent with elevated Cz P300 amplitudes measured in smokers in response to smoking-related images that have been previously reported in other smoking cue reactivity studies (Warren and McDonough 1999; McDonough and Warren 2001; Littel and Franken 2007; Luijten et al., 2016). There was a trend ($p = 0.06$) toward a main effect of condition for Pz P300 smoking cue reactivity, there were no significant differences in Pz P300 amplitudes between EOS and LOS, suggesting that while centro-parietal cortical reactivity may have increased overall in response to smoking imagery, the effect may have been more measurably localized at Cz. In contrast to oddball studies that measured elevated Fz reactivity to target smoking cues (Evans et al., 2013), EOS and LOS did not exhibit significant Fz P300 cue reactivity, which is consistent with Fz P300 findings from Engelmann and colleagues (2011). Overall, it is possible that three-stimulus oddball task performance, and differential sensitivity of LOS and EOS to tactile and visual smoking-related stimuli, may have influenced P300 amplitude and topography (Kennan et al., 2002; Luijten et al., 2016) in the current study.

One explanation for nicotine's effects on vulnerable maturational processes, overlapping with early stages of cigarette smoking, is through disruption of normative cholinergic system maturation and nicotinic cholinergic processing (Doura et al., 2008; Dwyer et al., 2009). Preclinical models of periadolescent nicotine exposure have shown evidence of cell loss and cell damage markers, as well as long-term alterations of cholinergic synaptic function, in maturing midbrain reward pathways (Slotkin, 2002; Abreu-Villaça et al., 2003b; Doura et al., 2008). Collectively, these alterations suggest a highly vulnerable early adolescent period that is receptive to 'misprogrammed' cellular maturation and synaptic function (Slotkin, 2002). Furthermore, nicotine-altered cholinergic activity and cellular damage affects midbrain regions involved in mediating nicotine reinforcing properties and reward (Trauth et al., 1999; Slotkin, 2002).

A speculative interpretation of elevated P300 smoking cue reactivity in EOS is that *initiation* of cigarette smoking during early adolescent maturation may influence cholinergic neurotransmission to accelerate the consolidation and stabilization of strong associations between rewarding effects of nicotine and smoking related stimuli. As such, the strength of these conditioned associations could be reflected in the higher craving and sensitized heightened P300 response to smoking-related imagery and objects measured in EOS.

One primary limitation of this study is the inability to examine sex-specific differences within the EOS and LOS groups in smoking cue reactivity. Sex was a nonsignificant covariate in current cue reactivity analyses; adolescent neuromaturation, however, occurs along different neurodevelopmental trajectories for males and females (Giedd et al., 1999). Future investigations should further explore the influence of sex-specific neuromaturation differences in craving and cue reactivity between early and late onset adult smokers. Another overall limitation for the present study was the small sample size although, importantly, groups were fairly well-matched for age, sex, education, and FTND scores and the fact that we observed significant differences in our *a priori* hypotheses means that we did not have a Type II error for these elements. Future investigations with larger EOS and LOS groups will determine if all three midline sites (Fz, Cz, and Pz) exhibit cue reactivity under additional experimental conditions (e.g. stress, prolonged nicotine deprivation) using oddball paradigms. Another acknowledged limitation is that the present study, like many others, relied on self-reports for establishing patterns of past and current nicotine (and other drug) use, mood, craving, and cognitive assessments. Self-report can sometimes be compromised by participant willingness to respond accurately as well as by overall estimation ability and perceived consequences to admissibility (Del Boca and Darkes 2003). Participants in the present study were assured that their responses to all questions and measures would be kept confidential and they were given sufficient time and targeted questions to help recall and report their past nicotine use, thus increasing our confidence in the reliability of the data.

3.1 Conclusions

In summary, P300 reactivity to salient smoking-related tactile and visual cues in adult smokers revealed different neurophysiological profiles for EOS and LOS participants that do not appear to be related to duration of smoking history. While the precise mechanism of this finding is unknown, preclinical evidence suggests that exposure to nicotine during early

maturation may result in compensatory neuroadaptations of cholinergic signaling systems. It is possible that similar neuroadaptations in EOS accelerated smoking-related sensitization in immature reward-related cholinergic pathways, consequently reinforcing associative reward circuitry activated by smoking cues. Differences in smoking cue reactivity profiles between EOS and LOS clearly have important implications for refining current smoking cessation interventions by emphasizing a more individualized approach to smoking-related cue exposure treatment.

4. Experimental Procedure

4.1. Participants

Ten early onset smokers (EOS; 5 women), ten late onset smokers (LOS; 5 women), and ten healthy non-smokers (HNS; 5 women) recruited from the Boston metropolitan area were included in this study conducted at McLean Hospital. Initial volunteer recruitment resulted in over 500 thorough screenings of cigarette smokers, and those who qualified by meeting stringent inclusion/exclusion criteria (described further below) were invited to participate. These stringent criteria were put in place to ensure that our hypothesized effects of early or late onset smoking on cue reactivity were not confounded by the effect of overlapping drug use. A primary factor that limited initial recruitment was that many smokers reported their onset of cigarette smoking behavior had overlapped, within one or two years, with the onset of marijuana (MJ) use. Early onset MJ use (use initiated prior to age 16), relative to late onset use (use initiated at or after age 16), has been associated with poor cognitive executive function in adult MJ users (e.g., Gruber et al., 2011; Gruber et al., 2012a, 2012b). To account for potential confounds related to co-occurring MJ initiation in this study, all smokers who had initiated any MJ use within five years of initiating cigarette smoking were excluded from study participation. This rigorous criterion eliminated the majority of initial volunteers but ensured that any adult smokers who did qualify for the study, and who had used MJ in the past, began their MJ exposure at least five years after initiating cigarette smoking. It also ensured that their MJ use would be considered late-onset use, which is associated with less pronounced effects on brain function (Gruber et al., 2011; Gruber et al., 2012a, 2012b). Overall, the ecological validity of the study findings apply to a subpopulation of smokers who maintain habitual smoking behaviors in adolescence for an extended period of time without initiating MJ use.

Though the sample size is modest, the EOS and LOS groups were extremely well-characterized for nicotine dependence with extremely limited prior drug use history. Twenty-three smokers met criteria and, of those, twenty (10 EOS and 10 LOS) chose to participate in the study. All participants were between the ages of 22–40 years old. Cigarette smokers were asked to report their age of cigarette smoking onset, specifically defined as when they began to regularly smoke cigarettes on a daily or near daily (approximately 5–7 days per week) basis. Smokers were then categorized as early onset if they reported daily or near daily smoking before age 16 or late onset if they reported daily or near daily smoking at or after age 16. All EOS and LOS currently smoked between 10–20 cigarettes daily at the time of recruitment into the present study, met DSM-IV criteria for nicotine dependence and scored 5 or greater on the Fagerström Test for Nicotine Dependence (FTND; Heatherton et

al., 1991). All HNS reported fewer than 5 lifetime uses of nicotine. Participants were clinically evaluated using the Structured Clinical Interview of DSM-IV Non-Patient Edition (SCID-1/NP) and were free of current Axis I diagnoses, neurological illness, and severe medical problems. Further exclusion criteria included current or recent (past 5 years) substance use disorders (other than nicotine), past diagnoses of bipolar disorder or schizophrenia, current or past psychoactive medication use, and prior episodes of loss of consciousness or head injury. One LOS and one EOS met criteria for past alcohol dependence (i.e., greater than, on average, 6 years ago). Group demographics, including education, body mass index, socioeconomic status, age of smoking onset, FTND score, duration of smoking exposure, and frequency of cigarette, alcohol, and marijuana use, are provided in Table 1.

In order to optimize conditions for measuring elevated craving and reactivity to smoking-related images (McClernon et al., 2005; Knott et al., 2008), all smokers were instructed to abstain from smoking for approximately 12 hours prior to laboratory arrival (Cortese et al., 2015) and were asked to report the time of their last cigarette when they arrived for their study visit. Participants reported that their last cigarette had been, on average, 11.5 hours $\pm$ 1.1 hours prior to their arrival. Compliance was assessed with carbon monoxide (CO) measurements (i.e., CO levels were < 12 ppm) in order for them to qualify for participation in the study. All smokers were compliant with maintaining overnight abstinence. All participants were also instructed to abstain from marijuana and alcohol use for approximately 12 hours prior to laboratory arrival, which was confirmed by CO (< 12 ppm) measurements and breathalyzer tests, respectively. Participants were further required to provide a negative drug (including THC) urine screen and have a negative pregnancy test. All procedures were approved by the McLean Hospital Institutional Review Board and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent and were compensated for their participation.

4.2. Procedures

Prior to the EEG cue reactivity session, all eligible participants completed a baseline clinical assessment battery of mood measures, including the Profile of Mood States (POMS; McNair et al., 1981); State-Trait Anxiety Inventory (Spielberger et al., 1983); Positive and Negative Affect Schedule (PANAS; Watson et al., 1988), Barratt Impulsiveness Scale (BIS-11; Patton et al., 1995); Hamilton Anxiety Scale (HAM-A; Hamilton, 1959); and Beck Depression Inventory (BDI-II; Beck et al., 1996) (Table 2). To assess potential baseline withdrawal and craving, all smokers also completed the Questionnaire on Smoking Urges (QSU-Brief; Tiffany and Drobes 1991) and the Wisconsin Smoking Withdrawal Scale (WSWS; Welsch et al., 1999) (Table 3).

4.3. EEG recording

Participants were fitted with a Waveguard™ 32-channel EEG/ERP electrode cap (ANT Neuro, Holland), with electrode sites positioned according to the 10–20 system. EEG was continuously recorded throughout the session using an ASA-Lab EEG/ERP 32 Channel Amplifier (ANT Neuro, Holland) and digitized at a rate of 256 Hz. An all-channel common average reference was used. To therefore ensure high signal quality across all channels, EEG

was recorded only with impedance at or below 5 k Ω . Two discrete electrodes were placed on the supra-orbital ridge and outer canthus of the left eye in order to record VEOG and HEOG, respectively. An electrode positioned on the Waveguard™ cap midway between frontal sites Fpz and Fz served as ground. Participants were seated in an electrically shielded chamber, and questionnaires and picture stimuli were presented on two separate computer monitors with corresponding response devices. Participants were also instructed about the importance of keeping head, face, and eye movements to a minimum throughout the session, and particularly during the picture presentation blocks. Two EOS participants (one male, one female) produced excess movement that interfered with accurate EEG data collection and were excluded.

4.4. Three-stimulus visual oddball task and mood and craving questionnaires

EEvoke software (ANT, Holland) was programmed to present all picture stimuli and send picture trigger information to the EEG acquisition and recording system. The present study, similar to EEG methodology previously reported by our group (Penetar et al., 2012), utilized a three-stimuli visual oddball paradigm for picture presentation with 60% frequent neutral images (e.g., chair, lamp), 20% target smoking-related target images (e.g., cigarette, lighter, ashtray etc.), and 20% arousing non-drug-related images (e.g., shark, dog baring teeth). The smoking-related images used in the study were chosen from free resources online and tested in a small pilot session with nicotine-dependent volunteers who reported moderate emotional arousal. Other investigations have also reported that smoking-related imagery is generally moderately arousing for cigarette smokers (Littel and Franken, 2007), thus an arousing non-drug-related image set in an oddball design would also need to be moderately arousing in order to control for non-specific arousal that might affect P300 amplitudes (Littel and Franken, 2011). The arousing image set, therefore, included images with moderate negative affect, such as a shark, a gun, and a dog baring its teeth. Both the arousal and neutral image sets used in this study were taken as subsets of the International Affective Picture System (Lang et al., 2008) and in-house resources (Nickerson et al., 2011) and previously tested in a study of cannabis-dependent adolescent patients (Nickerson et al., 2011). Pictures were presented via computer monitor to participants in pseudorandom order with repeated images (interstimulus interval = 1500 ms; duration = 750 ms; 96 neutral image presentations; 32 smoking-related image presentations; 32 arousing image presentations) and no two successive target picture presentations. Participants identified picture type by pressing one button for all images that were smoking-related and a different button for all images that were non-smoking-related (arousing and neutral). There was no practice session and ERPs were recorded during each block of picture presentations that were approximately 30 minutes apart. The first block took place 5 minutes after neutral object handling, and these events were collectively called the Neutral Condition. The second block of picture presentations took place 5 minutes after smoking-related object handling, and these events were collectively called the Smoking Condition. This order was not randomized for each participant because of potential carryover effects from handling smoking-related objects (Gray et al., 2008; Nickerson et al., 2011). The same three-stimuli picture set, presented pseudorandomly, and button press response contingency was utilized in both blocks. Picture stimulus response reaction times (RT), response accuracy, and errors of commission (failure to accurately identify target smoking-related images) and of omission (failure to respond to

image shown) for each block of neutral, arousing, and smoking-related image presentations were also calculated.

Participants also completed craving and mood self-report assessments at 4 distinct time points during the session: after neutral object handling, after the first picture presentation block, after smoking-related object handling, and after the second picture presentation block. Craving assessments included a subset of questions from a self-report questionnaire (developed in-house, 21 items; Nickerson et al., 2011) that examined craving: “How strong is your desire to smoke cigarettes,” “How strong is your desire to smoke right now?” and “How strong is your urge to smoke when your environment reminds you of it?” on a scale that ranged from 0 (*not at all*) to 100 (*most ever/strong desire*). Mood assessments from the self-report questionnaire included: “How anxious do you feel?”, “How upset do you feel?”, and “How happy do you feel?”.

4.5. Signal Processing and Analysis

All filtering and data analysis was performed offline using ASA-Lab software (ANT Neuro, Holland). Signal processing was concentrated on two EEG segments corresponding to continuous activity recorded during each block of picture presentations that followed exposure to neutral and to smoking-related tactile stimuli. Each EEG segment was band-pass filtered at 0.25–35 Hz with filter roll-off of 24 dB/octave. To correct the signal for repetitive EOG artifact and bursts of motion, a spatial principal component analysis (PCA)-based artifact correction algorithm was utilized. Additional automated artifact detection methods were performed on all EEG channels to remove any AC power line interference and detect peak-to-peak deflections that exceeded $\pm 40 \mu\text{V}$. These marked deflections and AC interference were consequently excluded from signal averaging. Each EEG segment was then signal averaged separately for neutral, smoking-related, and arousing stimulus picture trials, and further segmented into epochs of 100 ms pre-stimulus to 1000 ms post-stimulus for each stimulus category and to facilitate ERP analysis. These average waveforms for each stimulus category were then baseline corrected by subtracting the mean of the 100 ms window immediately preceding stimulus picture presentation from the remaining waveform activity. After artifact detection and elimination, the number of average trials that remained for each condition were: 29 target smoking-related images, 29 target arousing images, and 91 frequent neutral images for healthy non-smokers; 30 target smoking-related images, 31 target arousing images, and 93 frequent neutral images for early onset smokers; and 30 target smoking-related images, 31 target arousing images, and 94 frequent neutral images for late onset smokers.

The peak amplitude and latency of the P300 ERP component was measured relative to the 100 ms pre-stimulus baseline period, and calculated using a semiautomatic peak detection procedure as part of ASA-Lab software (ANT Neuro, Holland) with subsequent manual review and correction, if needed. The P300 peak was identified and marked in the signal averaged waveform of each electrode channel elicited by the target arousing, target smoking-related, and frequent neutral image stimuli as the largest positive peak between 250 and 550 ms after stimulus image onset (Houlihan et al., 1996; Mobascher et al., 2012; O’Donoghue et al., 2014).

4.6. Data Analysis

Demographic and baseline clinical assessment measures were analyzed using analyses of variance (ANOVAs), in addition to follow-up pairwise comparisons, to compare groups. Primary outcomes of the study included comparing smoking-related P300 amplitudes and peak latency at electrode midline positions Fz, Cz and Pz, as well as self-reported nicotine craving and mood, between Neutral and Smoking conditions. Though between-condition group comparisons were the primary focus of the investigation, within-condition group comparisons of midline P300 amplitudes and peak latency also were examined. Secondary outcomes were stimulus response RT, response accuracy, and errors of commission and of omission for each block of stimulus images measured between conditions. Primary (except for craving and mood) and secondary between-condition outcomes were analyzed using 2 (condition) $\times$ 3 (group) repeated measures (RM) analyses of covariance (ANCOVAs) controlling for sex (to account for evidence suggesting sex moderates smoking cue reactivity after exposure to smoking-related cues (Doran, 2014)) and duration of smoking exposure. Primary within-condition outcomes were analyzed using oneway ANCOVAs controlling for sex and smoking exposure. Craving and mood were analyzed using 4 (assessment time points) $\times$ 3 (group) repeated measures RM ANCOVAs also controlling for sex and duration of smoking exposure. The use of 4 assessment time points was to model session-wide group differences in subjected mood or craving reported after neutral object handling, after the first picture presentation, after smoking-related object handling, and after the second picture presentation. Planned *post hoc* pairwise comparisons to examine any group differences were performed only in the presence of a main-effect significant at the 0.05 level. Effect size *f* (ES) was calculated for significant main effects and interactions using G*power (Version 3.1.9.2; Faul, Erdfelder, Lang, and Buchner, Dusseldorf, Germany). Correlations of significant P300 reactivity observed in Fz, Cz, and Pz electrodes with clinical baseline measures and mood/craving ratings were assessed using nonparametric Spearman's rho correlation coefficients.

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Figure 1.

Grand mean average waveforms, specifically marking the P300 event-related potential, at the central (Cz) site are shown in response to smoking-related (black), arousing non-drug-related (gray), and neutral (dotted) image stimuli across Neutral (left) and Smoking (right) conditions. P300 smoking cue reactivity (peaks shown in box) was significantly different between conditions.

Figure 2.

Comparisons of significant group differences in grand mean average waveforms, specifically the P300 (peaks shown in box), at the central (Cz) site elicited by smoking cue reactivity in the Smoking condition are shown. (a) P300 smoking cue reactivity in early onset smokers (EOS; black line) compared to late onset smokers (LOS; gray line); (b) P300 smoking cue reactivity in EOS compared to healthy non-smokers (HNS; dotted); and (c) P300 smoking cue reactivity in LOS compared to HNS. ** $p < 0.01$ and * $p < 0.02$

**“Nothing Would Be Better Than Smoking
A Cigarette Right Now.”
(QSU-Brief; rate 1-5)**

Figure 3.

A scatterplot representing individual early onset and late onset smoker data and exhibiting a significant relationship between Cz P300 smoking cue reactivity in the Smoking condition and baseline craving is shown.

Table 1

Demographic and Substance Use Measures

	EOS (n=8)	LOS (n=10)	HNS (n=10)	<i>p</i>
Age	35.0 ± 2.5	31.2 ± 4.8	31.1 ± 3.9	ns
% female	50%	50%	50%	-
Education	10.9 ± 4.5	16.2 ± 2.5	11.4 ± 8.0	ns
FTND scores	6.0 ± 1.1	5.4 ± 1.0	-	ns
Age began daily/near daily smoking	13.0 ± 1.4	17.7 ± 1.2	-	0.000
Daily cigarettes smoked	18.1 ± 6.4	17.1 ± 5.5	-	ns
Smoking exposure (years)	22.0 ± 2.3	13.0 ± 4.6	-	0.001
Expired CO levels at study visit	8.8 ± 2.9	8.1 ± 3.3	2.3 ± 0.7	0.000 (EOS>HNS) (LOS>HNS)
Alcohol drinks/week	0.6 ± 0.9	6.7 ± 5.7	1.7 ± 1.7	0.01 (LOS>EOS) (LOS>HNS)
MJ use/week	0.4 ± 0.7	-	-	-

FTND Fagerstrom Test of Nicotine Dependence

CO carbon monoxide

MJ marijuana

Table 2

Clinical Mood Measures

	EOS (n=8)	LOS (n=10)	HNS (n=10)	<i>p</i>
HAM-A	0.6 ± 0.9	2.0 ± 1.9	0.8 ± 0.9	ns
BDI	4.4 ± 4.4	5.1 ± 5.3	0.6 ± 1.3	0.05 (LOS>HNS)
BIS Cognitive	22.3 ± 3.2	23.6 ± 2.6	22.7 ± 1.6	ns
Motor	26.1 ± 2.6	24.9 ± 2.5	21.5 ± 2.6	0.002 (EOS>HNS)
Non-planning	24.8 ± 2.1	25.5 ± 2.5	25.4 ± 2.2	ns
Total	73.1 ± 2.7	74.0 ± 3.0	69.6 ± 4.2	0.02 (EOS>HNS) (LOS>HNS)
POMS Tension	6.9 ± 6.1	9.0 ± 5.5	2.2 ± 2.1	.01 (EOS>HNS) (LOS>HNS)
Depression	2.8 ± 3.6	2.8 ± 2.5	0.6 ± 0.8	ns
Anger	3.3 ± 5.0	6.3 ± 5.7	0.4 ± 1.0	0.02 (LOS>HNS)
Vigor	16.1 ± 6.0	14.8 ± 8.5	16.9 ± 6.2	ns
Fatigue	3.6 ± 4.2	4.9 ± 2.9	1.9 ± 2.6	ns
Confusion	3.8 ± 3.3	6.0 ± 2.7	2.1 ± 1.2	0.01 (LOS>HNS)
PANAS Positive	31.4 ± 8.1	34.1 ± 7.5	35.5 ± 7.0	ns
Negative	13.3 ± 4.6	14.3 ± 3.4	11.0 ± 1.7	ns
STAI State	35.5 ± 9.1	37.8 ± 10.9	26.5 ± 4.6	0.02 (EOS>HNS) (LOS>HNS)
Trait	33.6 ± 8.9	36.2 ± 8.5	29.3 ± 6.7	ns

HAM-A Hamilton Anxiety Scale*BDI* Beck Depression Inventory*BIS* Barratt Impulsiveness Scale*POMS* Profile of Mood States*PANAS* Positive and Negative Affect Schedule

STAI State-Trait Anxiety Inventory

Table 3**Withdrawal and Craving Measures**

	EOS (n=8)	LOS (n=10)	<i>p</i>
WSWS Urge to Smoke	2.8 ± 0.8	2.9 ± 0.7	ns
Anxiety	1.7 ± 0.9	1.5 ± 1.1	ns
Irritability	1.5 ± 0.8	1.3 ± 0.9	ns
Sadness	1.1 ± 0.5	1.0 ± 0.8	ns
Increased appetite	1.9 ± 0.5	2.0 ± 0.8	ns
Difficulty concentrating	1.6 ± 0.9	1.3 ± 0.5	ns
Insomnia	1.9 ± 0.6	1.5 ± 0.8	ns
QSU Desire a cigarette right now	5.0 ± 0.0	4.8 ± 0.4	ns
Nothing better than smoking a cig right now	4.5 ± 0.8	2.7 ± 1.3	0.03
If possible I would smoke right now	4.8 ± 0.5	4.3 ± 1.3	ns
Could control things better if I could smoke	3.5 ± 1.5	2.3 ± 1.1	ns
All I want right now is a cigarette	4.5 ± 0.5	3.1 ± 1.2	ns
Urge for a cigarette	4.8 ± 0.7	4.6 ± 0.5	ns
Cigarette would taste good right now	4.4 ± 1.4	4.4 ± 1.0	ns
I would do almost anything for a cigarette	2.6 ± 1.6	1.8 ± 0.8	ns
Smoking would make me less depressed	1.9 ± 1.5	1.9 ± 1.3	ns
Going to smoke as soon as possible	4.6 ± 1.1	4.9 ± 0.3	ns
Total	44.0 ± 12.5	34.8 ± 5.0	0.05

WSWS Wisconsin Smoking Withdrawal Scale

QSU (QSU-Brief) Questionnaire on Smoking Urges

Table 4
Cz P300 Amplitudes Exhibited By Each Group To Stimulus Images in Each Condition

	Neutral Condition			Smoking Condition		
	Neutral	Arousing	Smoking-related	Neutral	Arousing	Smoking-related
EOS (n=8)	2.2 ± 1.7	6.6 ± 2.2	4.3 ± 0.8	2.3 ± 1.7	4.3 ± 1.8	7.1 ± 1.3
LOS (n=10)	0.3 ± 2.6	2.2 ± 3.1	4.5 ± 2.9	0.8 ± 1.5	2.0 ± 3.9	3.8 ± 1.9
HNS (n=10)	1.5 ± 3.4	4.6 ± 4.1	3.5 ± 2.7	2.2 ± 2.3	4.6 ± 2.8	4.0 ± 1.3