Psychophysiological and fMRI Investigations of Tobacco Cue Reactivity

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Dedication

Dedicated to the Engelmann family:

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Abstract

Development of new smoking-cessation therapies may be facilitated by identifying the neural basis of smoking-related emotional responses. In this dissertation, the affective consequences of cigarette smoking and abstinence were modeled in rats and humans using a potentiated-startle paradigm. In rats, repeated daily nicotine injections resulted in increased startle amplitude 2 h after nicotine exposure, which is consistent with the emergence of an anxiety-like withdrawal episode. In humans, startle responses to tobacco, pleasant, neutral, and unpleasant cues were measured in nonsmokers, nonabstinent smokers, and smokers who were 24 h into a 48 h abstinence period. Startle amplitude was potentiated during unpleasant cues in nonsmokers and abstinent smokers, but not in nonabstinent smokers, which suggests that smoking a cigarette reduced anxiety. Event-related brain potentials also suggested that abstinent smokers were more emotionally reactive than nonsmokers and nonabstinent smokers to both tobacco cues and unpleasant cues. An additional, functional magnetic resonance imaging study found that that two brain regions, the dorsal striatum and the anterior cingulate cortex, were involved in the expression of abstinent smokers' emotional responses to tobacco and unpleasant cues. These results suggest negative affect may be important in maintaining cigarette smoking and that the potentiated startle paradigm is an ideal model for preclinical and clinical studies of smoking-related emotional responses.

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CHAPTER 1: INTRODUCTION

Smoking is the leading preventable cause of disease and death in the United States. Despite widespread awareness of the adverse health consequences of smoking, it is estimated that 20 percent of the population smokes cigarettes (Centers for Disease Control and Prevention, 2007). Although most smokers seem to be aware of the health benefits of quitting and report a desire to quit, the number who successfully quit remains disappointingly low. Therefore, it is important to continue the search for more effective smoking cessation aids. Improved understanding of the neurobiological basis of tobacco addiction may provide new directions in this search (Dwoskin et al., 2009; Lerman et al., 2007). The goal of this dissertation is to describe the development of a laboratory model that can be used in the neuroscientific study of tobacco addiction and the development of new smoking-cessation therapies.

Neurobiology of Tobacco Addiction

Several reviewers have summarized current understanding of neural mechanisms that are related to addictive behavior (e.g., Goldstein & Volkow, 2002; Kalivas & Volkow, 2005; Koob & Le Moal, 2001, 2008; Koob & Volkow, 2010; T. E. Robinson & Berridge, 1993, 2003; Wise, 1988). In this section, I summarize the addiction literature in terms of how the results from previous studies can be expanded upon to facilitate treatment development.

Tobacco addiction is characterized by four classes of psychological phenomena, each of which is thought to be primarily mediated by a specific neural system. These are: 1) the positive affective consequences of smoking, 2) the negative affective

consequences of abstinence, 3) failure to exert inhibitory control over smoking urges, and 4) habitual or "automatic" smoking.

Positive affective consequences of smoking: Role of the mesocorticolimbic dopamine system

Although an individual's initial experience with smoking is typically unpleasant, characterized by dizziness, nausea, tremors, tension, and confusion, this reaction is quickly replaced by more desirable consequences that include euphoria, relaxation, and a sense of improved concentration (Foulds et al., 1997; Perkins et al., 1994). These appetitive aspects of tobacco intake are thought to be mediated by the effects of nicotine (the primary psychoactive ingredient of tobacco smoke) on the mesocorticolimbic dopamine system. The mesocorticolimbic dopamine system refers to a set of dopaminergic neurons with cell bodies in the midbrain ventral tegmental area (VTA) that send projections via the medial forebrain bundle to structures including the nucleus accumbens (ventral striatum), anterior cingulate cortex, and medial prefrontal cortex. This pathway is thought to mediate the rewarding consequences of all drugs of abuse (e.g., Di Chiara & Imperato, 1988). Indeed, animal models have shown that nicotine injection results in increased firing of VTA neurons (Rasmussen & Czachura, 1995; Schilstrom, Rawal, Mameli-Engvall, Nomikos, & Svensson, 2003) in rats and increased metabolic activity and dopamine release in the nucleus accumbens in rats (Di Chiara & Imperato, 1988; Pontieri, Tanda, Orzi, & Di Chiara, 1996) and nonhuman primates (Dewey et al., 1999). Blockade of dopaminergic transmission eliminates nicotine's ability to lower brain-reward thresholds measured using intracranial self-stimulation in

rats and to produce a conditioned-place preference (Pak et al., 2006), both of which are thought model nicotine's pleasurable consequences. Administration of dopamine receptor antagonists systemically (Corrigall & Coen, 1991) or directly into the nucleus accumbens (Corrigall, Franklin, Coen, & Clarke, 1992) or nicotinic acetylcholine receptor (nAChR) antagonists into the VTA (Corrigall, Coen, & Adamson, 1994) also blocks nicotine self-administration in rats, which supports the hypothesis that the mesocorticolimbic pathway is involved in the expression of nicotine's pleasurable consequences.

In humans, the effects of nicotine on dopamine release have been studied using position emission tomography (PET), which is a minimally-invasive procedure that maps the distribution of radioactively labeled molecules in conscious subjects. PET studies have shown that smoking a cigarette increases dopamine release in the ventral striatum (e.g., Brody, Olmstead et al., 2004) and that the amount of dopamine release correlates with smoking-induced mood improvement (Brody et al., 2009), which agrees with the findings from the animal models.

These findings implicate the mesocorticolimbic dopamine system as a potential target for stop smoking medications. Pharmacotherapies that reduce nicotine-induced activation of VTA dopamine neurons or dopamine release in the ventral striatum may be effective at reducing the subjective experience of pleasure that accompanies smoking, thereby reducing the likelihood of continued smoking. Possible medications include nAChR antagonists or partial agonists, which prevent nicotine-induced activation of VTA dopamine neurons and subsequent dopamine release in the nucleus

accumbens (Corrigall et al., 1994). In fact, the nAChR partial agonist varenicline has been shown to be effective in reducing positive affective consequences of smoking and to improve smoking cessation rates in clinical trials (R. West, Baker, Cappelleri, & Bushmakin, 2008). However, the number of smokers who successfully quit as a result of varenicline treatment remains relatively low (approximately 40% early in treatment, falling to approximately 20% after 1 year; Jorenby et al., 2006; Nides et al., 2006; Oncken et al., 2006), which underscores the importance of further studying the role of the mesocorticolimbic and other brain systems in tobacco addiction.

Negative affective consequences of abstinence: Role of the extended amygdala

Nearly all smokers report that unpleasant symptoms emerge when they are not smoking. This tobacco abstinence syndrome is characterized by intense urge or craving for cigarettes, anxiety, depressed mood, irritability, anger, restlessness, tension, difficulty concentrating, confusion, sleep disturbance, and increased appetite (e.g., American Psychiatric Association, 2000; Gritz, Carr, & Marcus, 1991; Hatsukami, Hughes, Pickens, & Svikis, 1984; Hughes, 1992, 2007; Hughes, Gust, Skoog, Keenan, & Fenwick, 1991; Hughes & Hatsukami, 1986; Hughes, Hatsukami, Pickens, & Svikis, 1984; Hughes, Keenan, & Yellin, 1989; Myrsten, Elgerot, & Edgren, 1977; Shiffman & Jarvik, 1976). During periods of protracted tobacco abstinence, several observable signs accompany self-reported abstinence symptoms. These include increased caloric intake and weight gain, decreases in heart rate, blood pressure, and circulating catecholamines, and increased skin temperature (e.g., R. M. Gilbert & Pope, 1982; Gritz et al., 1991; Hall, Ginsberg, & Jones, 1986; Hatsukami et al., 1984; Hughes, 1992;

Hughes et al., 1991; Hughes & Hatsukami, 1986; Myrsten et al., 1977). Most signs and symptoms of tobacco abstinence reach their peak severity within 48 h of smoking cessation and gradually decline over the course of 4-6 weeks, with the exception of weight gain and decreased heart rate, which appear to be permanent in many individuals who quit smoking (Gritz et al., 1991; Hall et al., 1986; Hughes, 1992, 2007; Hughes et al., 1991; Shiffman & Jarvik, 1976).

The negative affective nature of the tobacco abstinence syndrome is noteworthy, and this has lead to the hypothesis that smoking relapse is motivated by escape from unpleasant withdrawal symptoms (e.g., Baker, Piper, McCarthy, Majeskie, & Fiore, 2004; Koob & Le Moal, 2001; Poulos, Hinson, & Siegel, 1981; Solomon & Corbit, 1973; Watkins, Koob, & Markou, 2000; Wikler, 1973). Indeed, many studies have found evidence of a relationship between smoking relapse and the severity of craving and negative affect that emerge during abstinence (Allen, Bade, Hatsukami, & Center, 2008; Doherty, Kinnunen, Militello, & Garvey, 1995; Killen & Fortmann, 1997; Killen, Fortmann, Kraemer, Varady, & Newman, 1992; O'Connell & Shiffman, 1988; Piasecki, Fiore, & Baker, 1998; Piasecki et al., 2000; Pomerleau, Adkins, & Pertschuk, 1978; Shiffman, 1982, 1986; Shiffman et al., 1997; Shiffman et al., 1996; R. J. West, Hajek, & Belcher, 1989) and many smokers report that smoking a cigarette reduces anxiety (Parrott, 1993, 1995).

Negative affective consequences of tobacco withdrawal are thought to arise from two sets of neurobiological changes. One set of changes involves a reversal of nicotine-induced increases in reward sensitivity of the mesocorticolimbic dopamine

system (Epping-Jordan, Watkins, Koob, & Markou, 1998). The other set of changes involves the extended amygdala, a set of subcortical nuclei that include the central (CeA) and basolateral (BLA) nuclei of the amygdala, bed nucleus of the stria terminalis (BNST), and shell of the nucleus accumbens. The extended amygdala is involved in the expression of defensive behaviors, such as fear- and anxiety-like responses to aversive stimuli (Davis, 2000). This set of structures is also thought to be involved in the emergence of negative affect during nicotine withdrawal. In rats, the extended amygdala has been implicated in the expression of withdrawal-induced increases in anxiety-like behavior (George et al., 2007; Pandey, Roy, Xu, & Mittal, 2001; Tzavara, Monory, Hanoune, & Nomikos, 2002) and nicotine self-administration (George et al., 2007) and in withdrawal-induced decreases in the brain's sensitivity to rewarding electrical stimulation (Marcinkiewcz et al., 2009). In humans, functional magnetic resonance imaging (fMRI) has been used to detect greater amygdala activity in abstinent smokers than in nonabstinent smokers (Wang et al., 2007).

Knowledge of the role of the extended amygdala in tobacco withdrawal provides another route for the development of smoking-cessation therapies. Existing therapies such as nicotine replacement, clonidine, bupropion, and varenicline have been shown to reduce withdrawal-induced affective changes (e.g., Glassman, Jackson, Walsh, Roose, & Rosenfeld, 1984; Jorenby et al., 1999; R. West et al., 2008), but the neurobiological mechanisms by which these medications act are unknown. Given the importance of negative affect avoidance in smoking relapse, it is important to examine the role of the extended amygdala in the mechanisms of action of existing medications and to develop

new medications that target this system. Such medications may improve the efficacy of smoking cessation therapies at reducing affective signs of withdrawal and at facilitating successful abstinence.

Inhibitory control over smoking urges: Role of the prefrontal cortex

Smokers often find it difficult to inhibit their urge or craving to smoke in the presence of smoking-related cues or under conditions of stress (e.g., Shiffman et al., 1996). In animals, these situations have been modeled using cue-induced and stress-induced reinstatement of nicotine self administration after an extinction period. The anatomical substrates of these phenomena have not been studied as thoroughly in animals as the acute rewarding effects of nicotine and the aversive effects of withdrawal, but systemic pharmacological manipulations have shown that neurotransmitter systems such as corticotropin releasing factor (CRF; Bruijnzeel, Prado, & Isaac, 2009) and norepinephrine (NE; Chiamulera, Tedesco, Zangrandi, Giuliano, & Fumagalli, 2010) may be involved in cue- and stress-induced relapse. In humans, cue-induced craving has been shown to correlate with metabolic activity in the anterior cingulate gyrus, orbitofrontal cortex, and medial frontal cortex (e.g., Brody, Mandelkern et al., 2004; Brody et al., 2002; McClernon, Hiott, Huettel, & Rose, 2005; McClernon, Kozink, Lutz, & Rose, 2009; Stippekohl et al., 2010). These regions are involved in the expression of inhibitory control over behavior and error monitoring (e.g., Goldman-Rakic, 1995), which suggests that they may be involved in active inhibition of cue or stress induced smoking urges (Goldstein & Volkow, 2002; Koob & Volkow, 2010).

Studies of cue-induced relapse and stress-induced relapse suggest that the CRF and NE systems, possibly via their projections to the prefrontal cortex, may be promising targets for stop smoking medications. Because CRF and NE are also involved in the expression of negative affective withdrawal symptoms (e.g., George et al., 2007; Glassman et al., 1984), medications that target these systems may be especially efficacious remedies for individuals who smoke to reduce anxiety, be it via the reduction of withdrawal symptoms or a means of coping with stress. It appears that nearly all smokers are motivated by at least one of these two factors (Ikard, Green, & Horn, 1969; Ikard & Tomkins, 1973; Piper et al., 2004; Russell, Peto, & Patel, 1974; Shenassa, Graham, Burdzovic, & Buka, 2009), which underscores the importance of pursuing CRF-related, NE-related, and other affect-management therapies.

Habitual smoking: Role of the dorsal striatum

Many experienced smokers report that smoking becomes "automatic" or a "habit" and that their smoking behavior is performed, for the most part, in the absence of conscious cognitive control (Ikard et al., 1969; Ikard & Tomkins, 1973; Piper et al., 2004; Russell et al., 1974; Shenassa et al., 2009). The neurobiological basis of habitual smoking has not specifically been addressed in animals, most likely because it is impossible to differentiate between "automatic" and "consciously controlled" behavior in nonhumans. However, studies of rats and nonhuman primates have implicated the dorsal striatum (caudate nucleus and putamen) in the expression of simple, stimulus-response (S-R) habit learning (e.g., Balleine, Delgado, & Hikosaka, 2007; Jog, Kubota, Connolly, Hillegaart, & Graybiel, 1999). This has lead to the hypothesis that habitual

smoking in humans may be initiated via a system that includes dopaminergic projections from the substantia nigra to the dorsal striatum (Everitt et al., 2008; Everitt & Robbins, 2005). There is some evidence for dorsal striatal involvement in smoking. For example, PET studies have found that smoking a cigarette leads to increased dopamine release in the head of the caudate (Barrett, Boileau, Okker, Pihl, & Dagher, 2004). This suggests that dorsal striatal dopamine systems, in addition to the mesocorticolimbic system, are a potential target for stop-smoking medications. Further research into the role of the dorsal striatum in smoking behavior is therefore necessary.

Challenges in the study of smoking motivation:

The need for translational models

Human and animal models have provided considerable insight into the neurobiological basis of tobacco addiction. However, existing knowledge of the neurobiology of tobacco addiction has not advanced the development of new treatments as fast as might be expected. One reason for this slow progress is the lack of a good translational model between animals and humans. Most of the behavioral paradigms used to study addictive behavior in humans and nonhumans differ substantially, making it difficult to predict the efficacy of treatments in humans from the results of animal studies. Thus, it is important to develop improved cross-species models of tobacco addiction.

The problem of finding similar behavioral measures in humans and nonhumans is especially difficult when studying tobacco abstinence. In humans, abstinence severity is quantified using self-report measures of cigarette craving, anxiety, and other

withdrawal symptoms. In animals, abstinence severity is typically inferred from paradigms such as the elevated plus maze (Bhattacharya, Chakrabarti, Sandler, & Glover, 1995; Irvine, Cheeta, & File, 2001; Pandey et al., 2001), open field test (Tzavara et al., 2002), defensive burying test (George et al., 2007), conditioned place aversion (Suzuki, Ise, Tsuda, Maeda, & Misawa, 1996), and intracranial self-stimulation (Epping-Jordan et al., 1998), or by observing somatic signs of withdrawal (Malin et al., 1992). These behaviors do not have easily identifiable analogs in humans. Thus, it is important to develop a measure of the severity of withdrawal-induced affective changes that can be observed in both humans and nonhumans.

Potentiated startle:

A new translational model of tobacco addiction?

The startle reflex is a whole-body reflex that occurs in response to a sudden, intense stimulus, such as a loud noise burst. Startle has a common topography (a sudden jump or flinch, caused by simultaneous contraction of skeletal muscles) and neural circuit that is preserved across all mammalian species. Startle amplitude can be easily and noninvasively measured in both humans and nonhumans, making it an ideal candidate for translational research.

One characteristic of the startle reflex that is critical for the study of addiction is that startle amplitude is influenced by emotional states, increasing when elicited in the presence of aversive stimuli (*fear-potentiated startle*; Davis & Astrachan, 1978; Grillon & Davis, 1997; Schupp et al., 2004) and decreasing when elicited in the presence of appetitive stimuli (*pleasure-attenuated startle*; Grillon, Falls, Ameli, & Davis, 1994;

Schmid, Koch, & Schnitzler, 1995). The neural basis of this *emotion-modulated startle* effect has been thoroughly studied in rats (e.g., Kim & Davis, 1993; Walker & Davis, 1997b) and nonhuman primates (e.g., Antoniadis, Winslow, Davis, & Amaral, 2007; Davis, Antoniadis, Amaral, & Winslow, 2008), and is thought to be very similar in humans (Lang, Bradley, & Cuthbert, 1990). Neurons in the CeA and BNST that send axons to the nucleus reticularis pontis caudalis (nRPC), part of the brainstem startle circuit (Lee, López, Meloni, & Davis, 1996), are the final common pathway for emotion modulation of the startle reflex (Davis, Falls, Campeau, & Kim, 1993). Thus, the amygdala has been described as a "central fear system" (Davis, 2000 p. 214).

Nicotine withdrawal is an anxiety-like state, mediated in part by the extended amygdala (George et al., 2007; Marcinkiewcz et al., 2009; Panagis, Hildebrand, Svensson, & Nomikos, 2000; Pandey et al., 2001; Tzavara et al., 2002; Wang et al., 2007). Thus, potentiated startle should emerge during nicotine withdrawal. Indeed, a *withdrawal-potentiated startle* effect has been observed in rats during a single withdrawal episode after continuous exposure to nicotine for an extended period (7-28 days; Helton, Modlin, Tizzano, & Rasmussen, 1993; Helton, Tizzano, Monn, Schoepp, & Kallman, 1997; Rasmussen et al., 2000; Rasmussen, Czachura, Kallman, & Helton, 1996; Rasmussen, Kallman, & Helton, 1997). This is consistent with evidence provided by other animal models such as the defensive burying, elevated-plus maze, and open-field tests that suggests that nicotine withdrawal is anxiogenic in rats (Bhattacharya et al., 1995; George et al., 2007; Irvine et al., 2001; Pandey et al., 2001; Tzavara et al., 2002).

In the first studies of nicotine withdrawal-potentiated startle (Helton et al., 1993; Helton et al., 1997; Rasmussen et al., 2000; Rasmussen et al., 1996; Rasmussen et al., 1997), nicotine was continuously infused for several weeks, followed by a single, severe withdrawal episode. However, human smoking follows a more sporadic pattern, which results in the emergence of mild withdrawal symptoms several times per day (Parrott, 1993, 1995). Thus, it is important to study the effects of repeated, intermittent exposures to nicotine on withdrawal-potentiated startle in rats. In Chapter 2, I report the results from a study that demonstrated that withdrawal-potentiated startle emerges after several discrete exposures to nicotine.

In Chapter 3, I describe a study that extended the measure of withdrawal-potentiated startle described in Chapter 2 from rats to humans. I used a cue reactivity paradigm in which I measured emotion-modulated startle in response to tobacco, pleasant, neutral, and unpleasant cues in nonsmokers, nonabstinent smokers, and abstinent smokers who were 24 h into a 48-h abstinence period. Unlike the results from the study with rats, we did not observe withdrawal-potentiated startle in the absence of overt cues. However, we did observe greater fear-potentiated startle to unpleasant cues in abstinent smokers than in nonabstinent smokers.

Several other psychophysiological measures were also obtained in the emotion-modulated startle study. These include event-related potentials (ERPs) elicited by the startle probe (Chapter 3) and ERPs, facial muscle activity, and skin conductance responses elicited by the onset of the emotional and smoking-related cues (Chapter 4). Results from these psychophysiological measures suggest that abstinent smokers are

more emotionally reactive than nonabstinent smokers, especially to smoking-related and unpleasant cues. This set of results agrees with the findings of the withdrawal-potentiated startle study in rats and emotion-modulated startle in humans, and provides convergent validity of the startle results.

Because the cue reactivity paradigm appeared to be a valid measure of abstinence-induced emotional changes in humans and was consistent with the results of the rodent model, I completed an fMRI study using the same cue reactivity task. The goal of this study, which is presented in Chapter 5, was to provide an initial description of neurobiological mechanisms involved in the expression of the responses measured in our paradigm that can provide regions of interest for additional functional brain imaging studies in humans and for direct neural manipulations in rats. Several of the findings from this study agree with those from other fMRI studies of smokers. Furthermore, this study provides evidence for possible involvement of the dorsal striatum in abstinent smokers' emotional responses, which suggests that this structure may have an additional role in tobacco addiction beyond the expression of automatic smoking.

Finally, in Chapter 6, I synthesize the results from the individual studies and discuss their relevance for further development of translational models of tobacco addiction.

CHAPTER 2: POTENTIATED STARTLE AS A MEASURE OF THE NEGATIVE AFFECTIVE CONSEQUENCES OF REPEATED EXPOSURE TO NICOTINE IN RATS

Numerous signs and symptoms of psychological distress have been reported during tobacco abstinence in dependent smokers (Hughes et al., 1991; Hughes & Hatsukami, 1986). The negative affective consequences of tobacco withdrawal, such as anxiety, are likely due to withdrawal from nicotine, the primary addictive ingredient (e.g., Hughes, Hatsukami, Pickens, Krahn et al., 1984; R. J. West, Jarvis, Russell, Carruthers, & Feyerabend, 1984). Given the role that avoidance of these consequences plays in relapse to smoking (Piasecki et al., 1998; Piasecki, Kenford, Smith, Fiore, & Baker, 1997; Piasecki et al., 2000), it is important to identify neural mechanisms involved in nicotine withdrawal.

Studies of withdrawal from continuously infused nicotine have provided valuable insight into mechanisms involved in a single, severe nicotine withdrawal episode, but they are not well-suited for examining the multiple withdrawal episodes that occur as a result of intermittent, discrete nicotine exposures. Due to the intermittent nature of drug-taking behavior in the earliest stages of dependence, it is important to examine the effects of repeated withdrawal episodes across multiple acute drug exposures. Such investigations may lead to a better characterization of neural adaptations involved in the emergence of nicotine dependence (A. C. Harris & Gewirtz, 2005).

Recently, a procedure has been developed using the acoustic startle reflex to measure the anxiety-like aspects of repeated opiate withdrawals. Elevated acoustic startle responding provides a reliable, cross-species measure of fear and anxiety (Walker, Toufexis, & Davis, 2003). Moreover, potentiated startle has also been observed during withdrawal from drugs of abuse such as ethanol (Krystal et al., 1997; Rassnick, Koob, & Geyer, 1992) and opiates (A. C. Harris & Gewirtz, 2004; Kalinichev & Holtzman, 2003; Stine et al., 2001) in both human and animal subjects. Robust elevations in acoustic startle responding have also been reported in rats upon cessation of chronic, continuous nicotine infusion (Helton et al., 1993; Helton et al., 1997; Rasmussen et al., 2000; Rasmussen et al., 1996; Rasmussen et al., 1997). Harris, Hanes, and Gewirtz (2004) observed an escalation in the level of startle potentiation across daily withdrawals from morphine, suggestive of increasingly severe withdrawal episodes across repeated opiate exposures. The goal of the current study was to determine whether similar escalations in the severity of withdrawal could be observed across repeated nicotine injections.

Few studies have examined the effect of repeated withdrawals from discrete nicotine exposures, but there is evidence for increased anxiety-like behavior after withdrawal from daily acute nicotine injection in paradigms such as the elevated-plus maze, the social interaction test, and a drug-discrimination procedure (Bhattacharya et al., 1995; Cheeta, Irvine, Kenny, & File, 2001; C. M. Harris, Emmett-Oglesby, Robinson, & Lal, 1986; Irvine et al., 2001). These findings, along with striking similarities between other aspects of nicotine and opiate withdrawal syndromes in both

rats (Ise, Narita, Nagase, & Suzuki, 2000, 2002; Malin et al., 1992; Malin, Lake, Payne et al., 1996; Malin, Lake, Short et al., 1996; Malin, Lake, V, Cunningham, & Wilson, 1993) and humans (Hughes, Higgins, & Bickel, 1994) supports the prediction that an escalation in withdrawal-potentiated startle severity similar to that for opiates will be observed across multiple nicotine exposures.

In four experiments, spontaneous and precipitated withdrawal from repeated injections of nicotine were examined. Experiment I established the time course of spontaneous withdrawal, measured as potentiation of the startle reflex, and assessed the effects of nicotine replacement. Experiment II assessed the dose-dependence of nicotine withdrawal-potentiated startle. Experiment III examined whether clonidine, an α2-adrenergic receptor agonist that reduces morphine withdrawal-potentiated startle in rats (A. C. Harris & Gewirtz, 2004) and relieves nicotine withdrawal symptoms in humans (Glassman et al., 1984), would reduce nicotine withdrawal-potentiated startle. Experiment IV investigated whether potentiated startle would emerge during withdrawal precipitated by mecamylamine, a nonselective nAChR antagonist.

Due to the similarities between the injection protocol used in the current study and those used to investigate drug-induced sensitization of locomotor activity (for a review, see Stewart & Badiani, 1993), we included measures of the rats' activity levels at the beginning of each startle test session. Although not a standard measure of locomotor activity (due to the small size of the test chambers), activity readings were used to assess whether increases in activity levels immediately after nicotine injection would be observed across days, similar to the sensitization effects observed using

conventional measures of locomotor activity (Janhunen, Linnervuo, Svensk, & Ahtee, 2005).

Methods

Animals

Male albino Sprague-Dawley rats, obtained from Harlan (Indianapolis, IN, USA; Experiments I and II) and Charles River (Raleigh, NC, USA; Experiments III and IV), were used. Rats were housed in hanging metal cages in groups of four per cage and were maintained on a 12-h light-dark cycle (lights on at 8:00 a.m.) with food and water continuously available. Upon arrival in the colony, rats were allowed a 2-week acclimation period followed by 3 days of handling and habituation to subcutaneous injections (one daily saline injection, 1 ml/kg body weight). All rats weighed between 270 and 370 g at the start of testing, and all tests were run during the light phase of the light-dark cycle. All experimental procedures conformed to the Principles of Laboratory Animal Care (National Institutes of Health publication no. 8023, revised 1996) and Guidelines for the Humane Care and Use of Laboratory Animals of The Institutional Animal Care and Use Committee at the University of Minnesota.

Drugs

(–)-Nicotine hydrogen tartrate salt, clonidine, and mecamylamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). All drugs were dissolved in saline (0.9% w/v) and injected subcutaneously in a volume of 1 ml/kg body weight. All nicotine doses are expressed as the base and all other doses as the salt. The nicotine solution was titrated to a pH of approximately 7.1 using sodium hydroxide.

Testing apparatus and procedure

Startle reflex amplitude and activity were tested using a stabilimeter device that has been described previously (Rothwell, Thomas, & Gewirtz, 2009). Briefly, cage displacement proportional to the rat's movement was measured by a piezoelectric accelerometer in the absence of discrete stimuli (*activity trials*) and in response to startle-eliciting noise bursts (*startle trials*). Activity levels for each activity trial were defined as the mean peak-to-peak accelerometer voltage within a 200-ms accelerometer sample, and response amplitude for each startle trial was defined as the mean peak-to-peak voltage during the first 200 ms after onset of the startle stimulus. The startle stimulus consisted of a 50-ms (rise-decay <5 ms) filtered white noise (low pass: 22 kHz) delivered through a high frequency speaker (RadioShack Supertweeter, range 5–40 kHz, Model 40- 1310b) located 7 cm from the side of each cage at intensities of 95 or 105 dB. Each test session consisted of a 5-min period, during which activity levels were monitored every 10 s in the absence of startle stimuli, followed by a 20-min period of startle testing. Startle stimuli (20 at 95 dB and 20 at 105 dB) were presented at a fixed 30-s interval. The two intensity levels were presented in a pseudorandom order.

Baseline startle measurement

At the start of each experiment, all rats received 2 days of baseline startle measurement. On both days, all rats were injected with saline 1 h after the beginning of the startle test session. The first baseline test day was intended to habituate the rats to the startle testing procedure, and data from this session were not analyzed. The second

baseline test day (subsequently referred to as baseline) was used to place animals into treatment groups with approximately equal mean baseline startle amplitude.

Experiment I: Time course of spontaneous withdrawal

In this experiment, rats received multiple injections of a single nicotine dose to determine if an escalation in withdrawal-potentiated startle severity would be observed across multiple nicotine exposures. Beginning the day after baseline, rats in one group (n =12) received daily saline injections and rats in a second group (n = 12) received daily nicotine injections (0.25 mg/kg) for a total of 14 days. On Days 1, 7, and 14, the rats were given a startle test session 1 h before nicotine or saline injection (referred to as the *pretest*), and four startle test sessions beginning 5 min, 1 h, 2 h, 3 h, and 4 h after the injection (referred to as *posttests*; Figure 2-1a). Rats were returned to their home cages between startle test sessions. On all other days of the spontaneous withdrawal procedure, no startle test sessions were conducted; the only treatment was the nicotine or saline injection. On Day 15, a test was included to evaluate whether the potentiated startle effect observed on the previous day was due to nicotine deprivation. A subset of rats (n = 8 from each group) underwent a startle testing procedure that was identical to that on Days 1, 7, and 14 with the following exceptions: animals in the nicotine group received a second nicotine injection (0.25 mg/kg), and animals in the saline group received a second saline injection 10 min prior to the 2-h test session, and there were two additional posttest sessions, taking place 5 and 6 h after the first injection (Figure 2-1b).

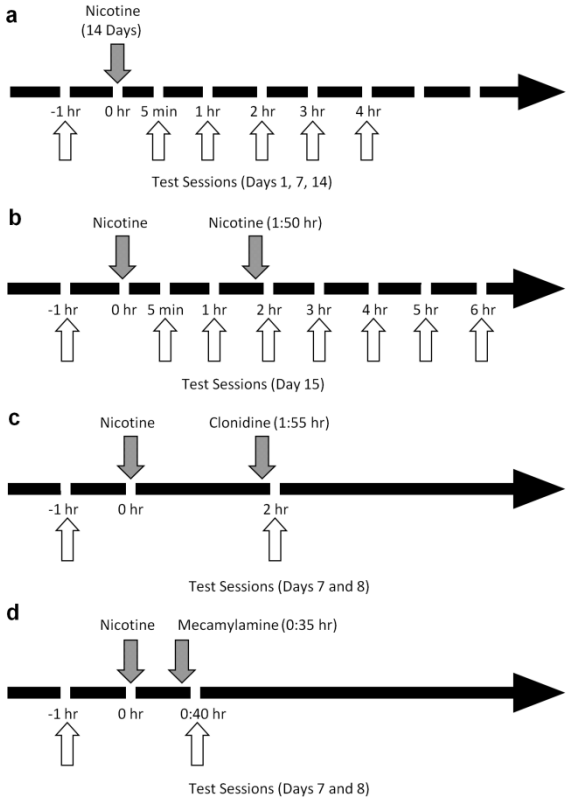

Figure 2-1. Experimental design. **a**) In Experiments I and II, rats were given daily nicotine injections (Experiment I: 0 or 0.25 mg/kg; Experiment II: 0, 0.125, 0.25, or 0.5 mg/kg) for 14 days. On Days 1, 7, and 14, test sessions took place 1 h prior to the injection and at multiple time points thereafter. **b**) On Day 15 in Experiments I and II, a second injection of nicotine (same dose as the first injection) took place 1:50 h after the first injection (i.e., at the time point where peak withdrawal-potentiated startle was observed on previous days). **c**) In Experiment III, rats were given daily nicotine injections for 8 days (0 or 0.5 mg/kg). On Day 8, clonidine (0, 10, or 15 µg/kg) was given 1:55 h after nicotine injection, and test sessions took place 1 h before and 2 h after the nicotine injection. **d**) In Experiment IV, rats were given daily nicotine injections for 8 days (0 or 0.5 mg/kg). On Day 8, mecamylamine (0 or 1 mg/kg) was injected 35 min after nicotine injection, and startle test sessions took place 1 h prior to and 40 min after nicotine injection. In all experiments, 0 h was defined as the time point of the first nicotine or saline injection. *Gray arrows* indicate injections. *White arrows* indicate the commencement of test sessions.

Experiment II: Dose-response evaluation

The purpose of Experiment II was to replicate the spontaneous withdrawal effect observed in Experiment I and to determine how different doses of nicotine modulate the withdrawal-potentiated startle effect. The procedures over 14 days were the same as those used in Experiment I, with the exception that there were four groups of rats (n = 8 per group), each assigned to a different nicotine dose (0, 0.125, 0.25, or 0.5 mg/kg/day).

Experiment III: Effect of clonidine on withdrawal-potentiated startle

The purpose of this experiment was to determine whether nicotine withdrawal-potentiated startle could be blocked by clonidine, an α2-adrenergic receptor agonist that has been shown to block affective signs of withdrawal in general (Smith & Aston-Jones, 2008) and opiate withdrawal-potentiated startle in particular (A. C. Harris & Gewirtz, 2004). Rats were assigned to groups that received either saline or nicotine (0.5 mg/kg) daily for 8 days (Figure 2-1c). On Day 6, all rats were given a startle test session 1 h prior to the nicotine or saline injection, to allow them to re-acclimate to the startle testing procedure. On Days 7 and 8, startle test sessions took place 1 h prior to and 2 h after the nicotine or saline injection. All animals received an additional injection 5 min prior to the start of the 2-h test session, which is where the peak withdrawal-potentiated startle effect was observed in Experiments I and II. On Day 7, this injection was saline for all animals. This test was conducted to make certain that animals were showing normal levels of withdrawal-potentiated startle 2 h after nicotine injection. On Day 8, animals within the saline and nicotine groups were assigned to three subgroups that were injected with clonidine (0, 10, or 15 μg/kg; *n*s = 9, 6, and 5 per subgroup).

Experiment IV: Precipitated withdrawal using mecamylamine

Because the first three experiments suggested that repeated nicotine exposure results in potentiated startle during spontaneous withdrawal, a final experiment examined whether a similar effect could be observed during precipitated withdrawal. Rats received daily injections of nicotine (0 or 0.5 mg/kg) for 8 days. On Day 6, all rats were given a startle test session 1 h prior to the nicotine or saline injection, to allow

them to re-acclimate to the startle testing procedure. On Days 7 and 8, startle test sessions took place 1 h prior to and 40 min after nicotine injection (Figure 2-1d), so that posttest startle amplitude during precipitated withdrawal could be measured before the emergence of spontaneous withdrawal-potentiated startle that was observed at later time points during Experiments I and II (cf. A. C. Harris & Gewirtz, 2004). Five minutes prior to the start of the posttest session on Day 7, all animals were injected with saline. On Day 8, half of the animals within each nicotine dose were injected with saline (n = 8 per nicotine dose), and the other half were injected with mecamylamine (1 mg/kg; n = 8 per nicotine dose).

Statistical analysis

As in previous studies (A. C. Harris, Atkinson, Aase, & Gewirtz, 2006; A. C. Harris & Gewirtz, 2004; A. C. Harris et al., 2004), the 105-dB startle stimuli resulted in greater startle amplitude than did the 95-dB startle stimuli, but there was no significant effect of startle stimulus intensity on the magnitude of startle potentiation (data not shown; Walker & Davis, 2002). Thus, startle amplitude was averaged across all 40 trials during the test session, creating a single startle amplitude score for each session. Similarly, a single activity score was created for each test session by averaging across all 30 activity readings measured during the first 5 min of the session. To verify that there were no significant between-groups differences in baseline or pretest startle amplitude, these data were subjected to a nicotine dose x test day analysis of variance (ANOVA), with group as a between-subjects factor and test day as a within-subjects factor.

In all experiments, withdrawal-potentiated startle was quantified as the percent change in startle amplitude between each day's pretest and posttest sessions (Walker & Davis, 2002). Analogous percent change scores were calculated for activity levels. Animals with percent change scores in startle or activity greater than three standard deviations from the population mean were considered outliers and excluded from analyses (Johnson & Wichern, 2002).

For Experiments I and II, percent change in startle and percent change in activity were analyzed separately using the general linear models procedure in SYSTAT 12 (SYSTAT Software, Inc., Chicago, IL, USA). For all tests, the criterion for significance was set at $p < .05$. Multivariate test statistics (Wilks λ and its approximate F statistic) were used to test the significance of all effects involving repeated measures because multivariate tests do not require the assumption of a spherical covariance matrix across all levels of a repeated measure (Johnson & Wichern, 2002; Maxwell & Delaney, 1990). In Experiments III and IV, planned contrasts were used to test the effect of clonidine or mecamylamine on withdrawal-potentiated startle. These contrasts are based on the results of previous experiments assessing the effect of clonidine on opiate withdrawal potentiated startle (A. C. Harris & Gewirtz, 2004).

Results

Experiment I

This experiment examined the time course of spontaneous withdrawal-potentiated startle from a daily dose of nicotine (0.25 mg/kg). Dose x test day ANOVA was used to evaluate whether there were any differences in startle amplitude across the

baseline day and pretests on Days 1, 7, and 14 of nicotine injection. The main effects of dose [$F(1,22) < 1$, $p > .1$] and day [$F(3,20) = 2.23$, $p > .1$], and the dose x day interaction [$F(3,20) < 1$, $p > .1$] were not significant (see Table 2-1).

Table 2-1. Mean ± SEM baseline and pre-injection startle amplitude (arbitrary units)

Nicotine dose (mg/kg)	Clonidine dose (μg/kg)	Mecamylamine dose (mg/kg)	Baseline	Day 1	Day 6	Day 7	Day 8	Day 14
Experiment I								
0			5.64 ± 0.54	5.09 ± 0.56		6.09 ± 0.77		6.61 ± 0.76
0.25			5.72 ± 0.25	5.72 ± 0.38		5.64 ± 0.41		6.04 ± 0.35
Experiment II								
0			7.52 ± 1.42	5.80 ± 1.04		6.97 ± 1.33		8.09 ± 1.17
0.125			6.63 ± 1.38	6.25 ± 1.51		6.76 ± 1.34		7.92 ± 1.69
0.25			6.74 ± 0.67	6.42 ± 0.45		8.42 ± 0.64		8.80 ± 1.04
0.5			7.44 ± 1.75	6.96 ± 1.16		8.39 ± 1.88		8.00 ± 1.40
Experiment III								
0	0		5.78 ± 0.87		7.14 ± 0.96	5.80 ± 0.74	5.04 ± 0.64	
0	10		4.13 ± 0.55		4.76 ± 0.73	4.91 ± 0.90	4.26 ± 1.01	
0	15		5.18 ± 1.45		5.58 ± 0.99	4.36 ± 0.47	4.53 ± 0.76	
0.5	0		6.21 ± 0.82		7.56 ± 0.96	6.28 ± 0.58	5.39 ± 0.58	
0.5	10		3.78 ± 0.80		4.31 ± 0.72	3.29 ± 0.23	2.96 ± 0.28	
0.5	15		4.22 ± 0.71		5.13 ± 0.77	4.40 ± 0.72	4.45 ± 0.82	
Experiment IV								
0	0		5.21 ± 0.90			6.75 ± 1.14	6.76 ± 1.21	
0	1		4.08 ± 0.65			4.96 ± 0.89	4.61 ± 0.78	
0.5	0		4.25 ± 0.56			5.63 ± 0.56	5.12 ± 0.46	
0.5	1		4.95 ± 0.46			5.82 ± 0.48	4.94 ± 0.33	

Examination of percent change in startle amplitude from pretest to posttest confirmed that startle responding escalates over repeated exposures to nicotine (Figure 2-2, left column; significant nicotine dose x day x test session interaction [$F(8,15) = 2.88$, $p < .05$, $\lambda = .39$]). Follow-up dose x test session ANOVAs within each day revealed significant dose x test session interactions on Days 7 [$F(4,19) = 5.56$, $p < .01$, $\lambda = .46$] and 14 [$F(4,19) = 16.32$, $p < .001$, $\lambda = .23$], but not on Day 1 [$F(4,19) = 2.23$, $p = .10$, $\lambda = .68$]. These interactions were reflective of differences in the shape of startle amplitude vs. time curves between the two groups, with significant startle potentiation in the nicotine group, compared to the saline group, during the 2-h test session on Days 7 [$F(1,22) = 4.49$, $p < .05$] and 14 [$F(1,22) = 8.34$, $p < .01$]. There was also a significant between-group difference during the 5-min test on Day 14 [$F(1,22) = 4.56$, $p < .05$], where startle magnitude was lower in the nicotine group than in the saline group. Presumably, elevated startle magnitude at this time point in the saline group was due to stress caused by the subcutaneous injections (similar to shock-induced sensitization of startle; see Davis, 1989), and nicotine reduced this effect, which is consistent with the hypotheses that nicotine is capable of reducing acute stress effects in rats (Acri, 1994).

Activity levels increased immediately after nicotine injection on Days 7 and 14, suggestive of an escalation in nicotine-induced activity across days, similar to the escalation in withdrawal-potentiated startle (Figure 2-2, right column). ANOVA for activity readings revealed a significant dose x day x test session interaction [$\lambda = .30$, $F(8,15) = 4.41$, $p < .01$], and follow-up dose x time point ANOVAs at each day found significant two-way interactions on Days 7 [$F(4,19) = 3.32$, $p < .05$, $\lambda = .59$] and 14

[$F(4,19) = 4.60$, $p < .01$, $\lambda = .51$], but not on Day 1 [$F(4,19) < 1$, $p > .1$, $\lambda = .84$]. These interactions were the result of significant effects of nicotine dose during the 5-min test session on Days 7 [$F(1,22) = 13.19$, $p < .01$] and 14 [$F(1,22) = 25.82$, $p < .001$]. The effect of nicotine dose was not significant at any other time point on Days 7and 14.

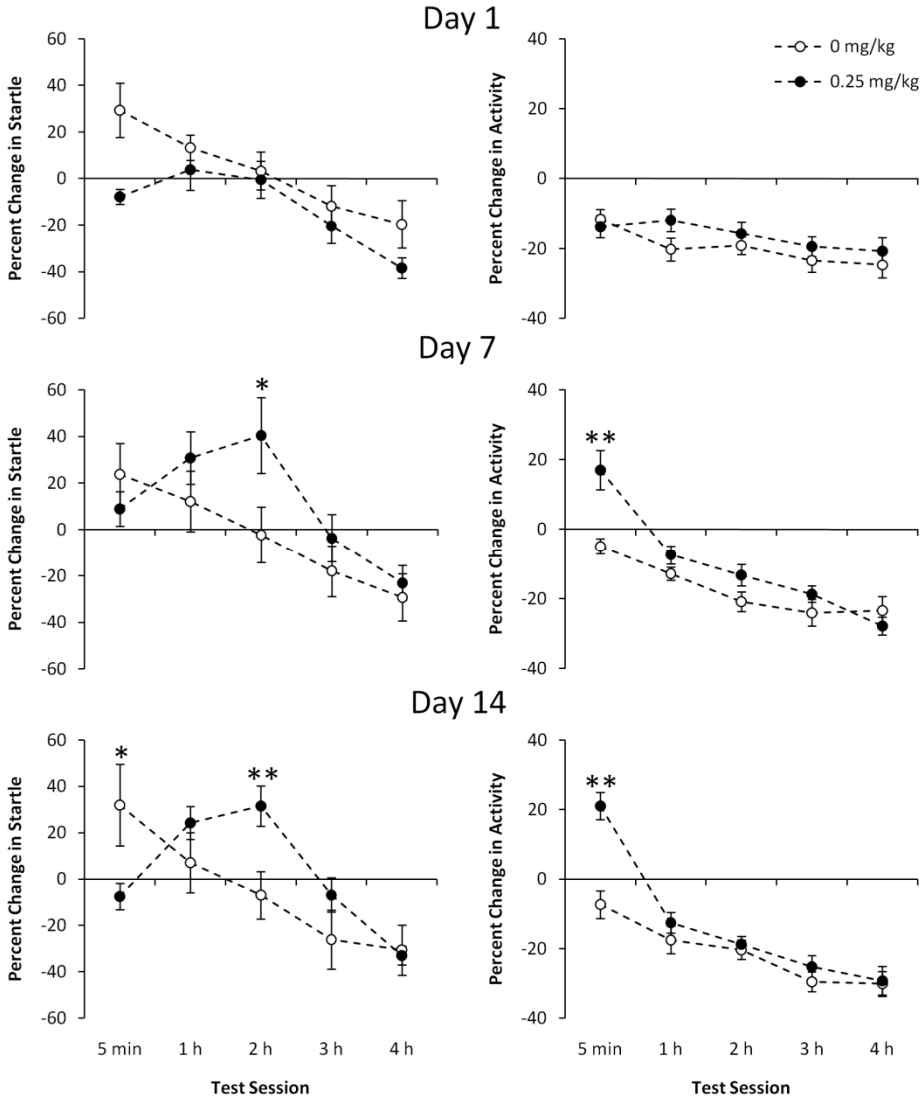

Figure 2-2. Daily nicotine injections (0 or 0.25 mg/kg) result in an escalation of withdrawal-potentiated startle severity across days and increased activity immediately after the nicotine injection. Data shown depict percent change in startle amplitude (*left column*) and activity levels (*right column*) from the same-day pretest at 5 min, 1 h, 2 h, 3 h, and 4 h after nicotine injection. *Asterisks* indicate a significant effect of nicotine dose at that time point (* $p < .05$, ** $p < .01$). $n = 12$ per nicotine dose.

On Day 15, a second nicotine injection temporarily reversed withdrawal-potentiated startle, delaying its peak from the 2- to the 3-h test session (Figure 2-3, upper panel). This result was supported by a significant dose x time point interaction [$F(6,9) = 16.98$, $p < .001$, $\lambda = .08$], with a significant effect of dose during the 1-h test session [$F(1,14) = 6.22$, $p < .05$], no significant effect of dose during the 2-h test session [$F(1,14) = 2.95$, $p > .1$], and re-emergence of the significant dose effect during the 3-h [$F(1,14) = 14.16$, $p < .01$] and 4-h [$F(1,14) = 8.08$, $p < .05$] test sessions. For activity, there was evidence of an increase after both the first and second nicotine injections (Figure 2-3, lower panel). The main effect of dose was significant [$F(1,14) = 5.80$, $p < .05$], but the main effect of test session [$F(6,9) = 2.39$, $p > .1$, $\lambda = .39$] and the dose x test session interaction [$F(6,9) = 1.89$, $p > .1$, $\lambda = .44$] were not significant. Importantly, follow-up tests revealed a significant effect of nicotine dose on activity during the 5-min [$F(1,14) = 9.05$, $p < .01$] and 2-h [$F(1,14) = 11.44$, $p < .01$], but not the 1-h [$F(1,14) = 2.80$, $p > .1$] test sessions.

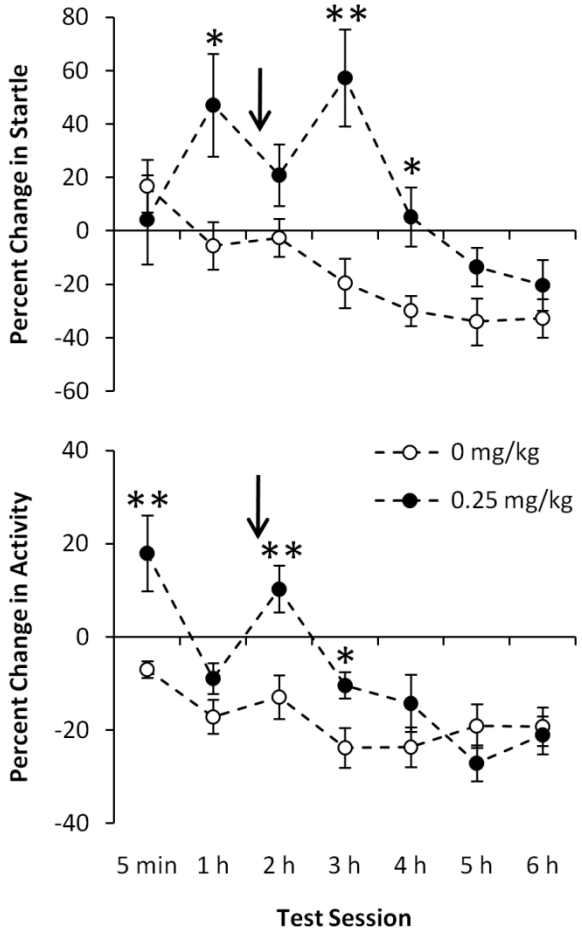

Figure 2-3. A second nicotine injection, administered immediately prior to the time point where peak withdrawal-potentiated startle was observed on previous days in Experiment I, delays the peak of withdrawal-potentiated startle to a later time point (*top panel*) and partially reinstates increased activity levels observed immediately after the first nicotine injection (*bottom panel*). *Arrows* indicate the timing of the second nicotine injection (1:50 after the first nicotine injection). *Asterisks* indicate a significant effect of nicotine dose at that time point (* $p < .05$, ** $p < .01$). $n = 8$ per nicotine dose.

Experiment II

Data from one animal in the 0.125 mg/kg nicotine group were excluded from analyses as an outlier. As in Experiment I, analysis of baseline and pretest startle amplitude found no evidence of a significant main effect of nicotine dose [$F(3,27) < 1$, $p > .1$] or dose x test day interaction [$F(9,60) = 1.40$, $p > .1$], indicating that there were no between-groups differences in startle amplitude prior to nicotine injection on any test

day. However, the main effect of test day was significant [$F(3,25) = 9.81$, $p < .001$; see Table 2-1]. Use of percent change scores from each test day's pre-injection startle amplitude allowed for assessment of withdrawal-potentiated startle independent of day-to-day variations in baseline startle amplitude (Walker & Davis, 2002).

As expected, analysis of percent change in startle from pretest to posttest yielded no significant evidence of withdrawal-potentiated startle on Day 1, and there were dose-dependent increases in startle amplitude during spontaneous withdrawal on Days 7 and 14 (Figure 2-4, left column), as evidenced by a significant dose x day x test session interaction [$F(8,22) = 3.65$, $p < .01$, $\lambda = .43$], and significant dose x test session interactions on Days 7 [$F(4,26) = 4.69$, $p < .01$, $\lambda = .58$] and 14 [$F(4,26) = 5.88$, $p < .01$, $\lambda = .52$], but not on Day 1 [$F(4,26) = 1.20$, $p > .1$, $\lambda = .84$]. Although these results were consistent with Experiment I in demonstrating an emergence of withdrawal-potentiated startle in the Days 7 and 14 tests, the effect appeared to be less robust on Day 7 in this experiment. Thus, significant effects of nicotine dose were observed during the 1-h [$F(1,29) = 4.99$, $p < .05$], 3-h [$F(1,29) = 8.11$, $p < .01$], and 4-h [$F(1,29) = 10.96$, $p < .01$] test sessions on Day 14. The dose effect during the 2-h test session, where peak withdrawal-potentiated startle was observed in Experiment I, approached significance on Day 14 [$F(1,29) = 3.51$, $p = .07$] but not on day 7 [$F(1,29) = 2.64$, $p > .1$], suggesting some variability in the rate of development of this phenomenon as a function of the number of nicotine exposures.

Experiment II also provided evidence that the increase in activity observed immediately after nicotine injection on Days 7 and 14 is dose-dependent (Figure 2-4,

right column). This result was supported by a dose x day x time point interaction that approached significance [$F(8,22) = 2.33$, $p = .07$, $\lambda = .55$], and significant dose × time point interactions on Days 7 [$F(4,26) = 9.39$, $p < .001$, $\lambda = .41$] and 14 [$F(4,26) = 7.44$, $p < .001$, $\lambda = .47$], but not on Day 1 [$F(4,26) = 2.11$, $p > .1$, $\lambda = .76$]. These significant interactions were the result of significant effects of dose during the 5-min test on Days 7 [$F(1,29) = 19.17$, $p < .001$] and 14 [$F(1,29) = 23.28$, $p < .001$], but at no other time point.

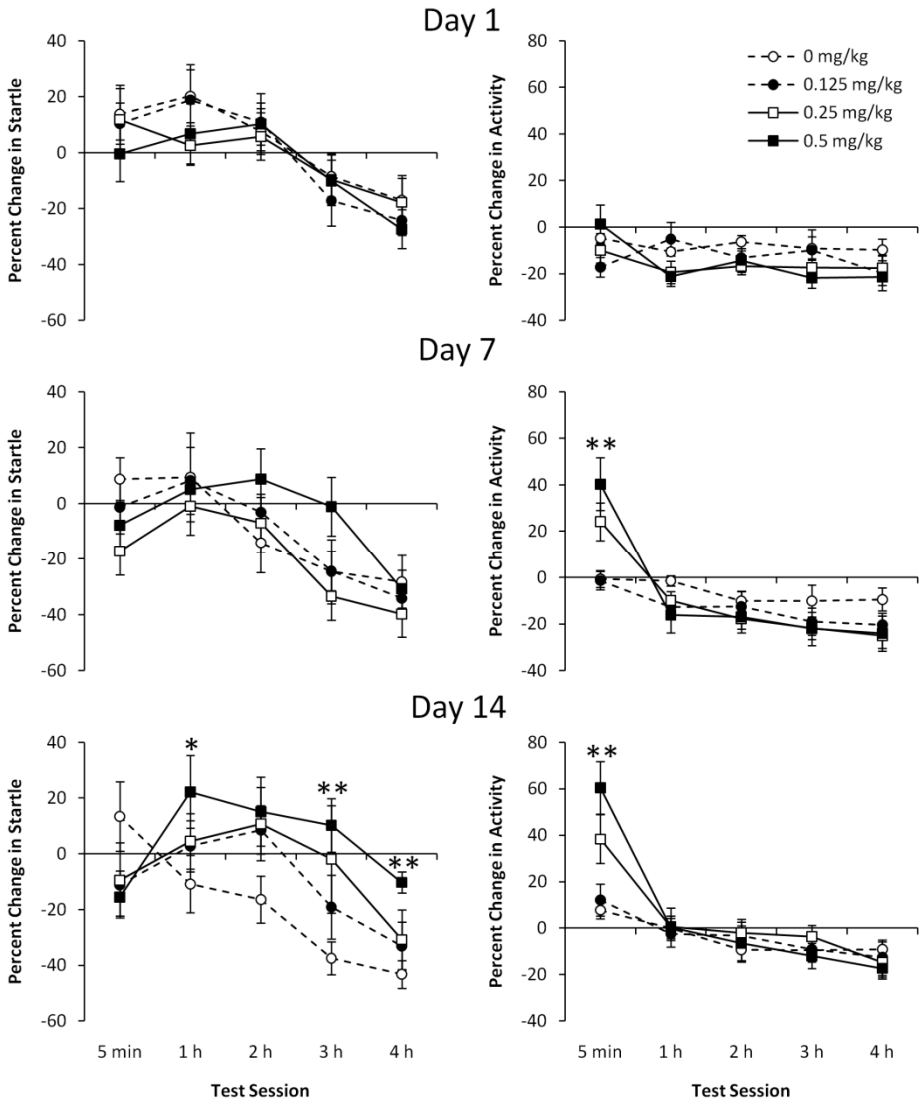

Figure 2-4. The magnitude of nicotine withdrawal-potentiated startle (*left column*) and nicotine-induced increases in activity (*right column*) is dose-dependent. In Experiment II, rats were injected with nicotine (0, 0.125, 0.25, or 0.5 mg/kg per day) for 14 days, and test sessions took place on Days 1, 7, and 14. Data depict the mean percent change in startle amplitude (*left column*) and activity levels (*right column*) from the same-day pretest during each post-injection test session. *Asterisks* indicate a significant effect of nicotine dose at that time point (* $p < .05$, ** $p < .01$). *n*s = 8, 7, 8, and 8 for the 0-, 0.125-, 0.25-, and 0.5-mg/kg groups, respectively.

Experiment III

Experiment III examined whether clonidine would dose-dependently reduce withdrawal-potentiated startle from 0.5 mg/kg nicotine. Nicotine dose x day ANOVA

found no significant main effect of dose [$F(1,38) < 1$, $p > .1$] or dose x day interaction [$F(3,36) < 1$, $p > .1$] on baseline and pretest startle amplitude, but there was a significant main effect of day [$F(3,36) = 11.95$, $p < .001$; see Table 2-1]. On Days 7 and 8 of nicotine injection (0 or 0.5 mg/kg), withdrawal-potentiated startle was assessed 2 h after injection. On Day 7, rats injected with 0.5 mg/kg had significantly greater percent change in startle amplitude than rats injected with 0 mg/kg nicotine [0mg/kg: M = -2.12%, SD = 5.85%; 0.5 mg/kg: M = 25.59%, SD = 5.50%; dose effect $F(1,38) = 11.91$, $p < .001$]. On Day 8, planned contrasts in the 0 μg/kg clonidine groups confirmed the presence of withdrawal-potentiated startle following the 0.5 mg/kg nicotine injection [Figure 2-5; $F(1,34) = 17.68$, $p < .001$]. As expected, clonidine dose-dependently reduced startle amplitude in the 0.5 mg/kg nicotine group [linear trend of clonidine dose: $F(1,34) = 9.27$, $p < .01$], but not in the 0 mg/kg nicotine group [linear trend of clonidine dose: $F(1,34) < 1$, $p > .1$].

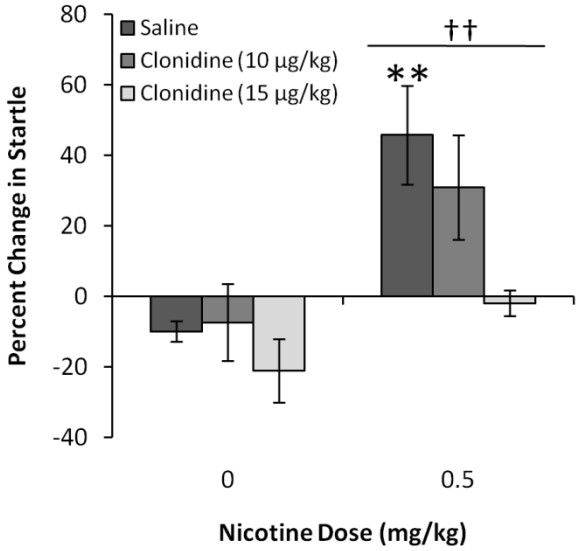

Figure 2-5. Clonidine dose-dependently decreases nicotine withdrawal-potentiated startle. Data shown are the percent change in startle on Day 8 from the pre-nicotine test session to the 2-h post-nicotine test session. Immediately prior to the post-nicotine test session, rats assigned to each level of nicotine dose (0 or 0.5 mg/kg) were injected with clonidine (0, 10, or 15 µg/kg). ** Indicates a significant difference between the group receiving 0 mg/kg nicotine followed by 0 mg/kg clonidine and the group receiving 0.5 mg/kg nicotine followed by 0 mg/kg clonidine (planned contrast, $p < .01$). †† Indicates a significant linear trend of clonidine dose within the 0.5 mg/kg nicotine groups (planned contrast, $p < .01$). *n*s = 9, 6, and 5 for each nicotine dose for the 0, 10, and 15 µg/kg clonidine doses, respectively.

Experiment IV

Experiment IV examined whether mecamylamine-precipitated withdrawal could be observed after 7 days of nicotine injection. Data from one animal in the 0.5 mg/kg nicotine-0 mg/kg mecamylamine group were excluded from analyses as an outlier. Also, technical difficulties resulted in the loss of Day 6 pre-injection startle data for 16 animals. Thus, Day 6 (which was only used to re-habituate rats to the startle testing procedure) was not included in the nicotine dose x day ANOVA on baseline and pretest startle amplitude. This ANOVA found no significant main effect of dose [$F(1,29) < 1$, $p > .1$] or dose x day interaction [$\lambda = .94$, $F(2,28) < 1$, $p > .1$], but there was a significant main effect of day [$\lambda = .74$, $F(2,28) = 4.94$, $p < .05$; see Table 2-1].

Analysis of percent change in startle from pretest to posttest found that, as expected, the early test session (40 min following 0 or 0.5 mg/kg nicotine) did not reveal significant withdrawal-potentiated startle on Day 7 [0 mg/kg: $M = 5.70\%$, $SD = 9.82\%$; 0.5 mg/kg: $M = 24.67\%$, $SD = 6.51\%$; dose effect $F(1,29) = 2.52$, $p > .1$]. On Day 8, there was also no evidence of nicotine withdrawal-potentiated startle in any of the four groups of rats [Figure 2-6; all Fs$(1,27) < 1$, all ps $> .1$].

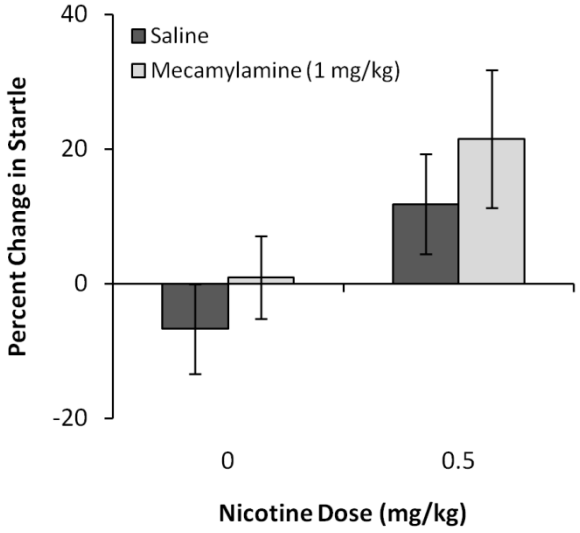

Figure 2-6. Mecamylamine-precipitated withdrawal-potentiated startle is not evident. Data shown are the percent change in startle on Day 8 from the 40-min post-nicotine test session. Immediately prior to the post-nicotine test session, rats assigned to each level of nicotine dose (0 or 0.5 mg/kg) were injected with mecamylamine (0 or 1 mg/kg). ns = 8 and 7 for the 0 and 0.5 mg/kg nicotine doses in the groups injected with 0 mg/kg mecamylamine, and ns = 8 and 8 for the 0 and 0.5 mg/kg nicotine doses in the groups injected with 1 mg/kg mecamylamine, respectively.

Discussion

Emergence of negative affective withdrawal symptoms is thought to be an important factor in the transition from initial drug use to dependence and addiction (Koob & Le Moal, 2001; Solomon & Corbit, 1973). Thus, animal models of these processes may lead to improved understanding of neural mechanisms involved in drug

dependence. The withdrawal-potentiated startle procedure developed in the current study, which provides a methodology for measuring anxiety-like withdrawal episodes from repeated exposures to nicotine, may provide a valuable tool for the development of such models.

Several findings from the current study support the validity of withdrawal-potentiated startle as a measure of negative affective aspects of nicotine withdrawal. First, our findings agree with observations that potentiated startle emerges during withdrawal from chronic, continuously infused nicotine (Helton et al., 1993; Rasmussen et al., 2000; Rasmussen et al., 1996; Rasmussen et al., 1997). This suggests that the withdrawal episodes produced in our studies – those that emerge after repeated, intermittent nicotine exposure – are similar to those observed after continuous infusion, which raises the possibility that intermittent nicotine exposure paradigms can be used to study the initial development of the same components of withdrawal that persist through long-term, continuous nicotine exposure (A. C. Harris & Gewirtz, 2004). Second, withdrawal-potentiated startle is temporarily blocked by re-administration of nicotine, which is also consistent with observations from studies of withdrawal from chronic, continuous nicotine (Helton et al., 1993). This observation is also clinically relevant in that relief from the anxiety-related consequences of abstinence constitutes a critical motivational factor in compulsive smoking behavior (Piasecki et al., 1997). Third, withdrawal-potentiated startle appears to be dose-dependent, with both larger increases and later peaks at higher doses. Fourth, nicotine withdrawal-potentiated startle is blocked by clonidine, an α2-adrenergic receptor agonist that has been shown to block

withdrawal-potentiated startle from morphine (A. C. Harris & Gewirtz, 2004) and to reduce affective symptoms and craving in humans withdrawing from tobacco (Glassman et al., 1984). Blockade of withdrawal-potentiated startle by clonidine is also consistent with the hypothesis that increases in central noradrenergic function are involved in the expression of withdrawal from drugs of abuse (Smith & Aston-Jones, 2008).

It should be noted that not all studies of withdrawal from chronic, continuous nicotine exposure have observed increases in baseline startle responding (Acri, Brown, Saah, & Grunberg, 1995; Acri, Grunberg, & Morse, 1991; Jonkman, Risbrough, Geyer, & Markou, 2008). Jonkman et al. (2008) reported that withdrawal from nicotine after 28 days of chronic infusion via osmotic minipump did not potentiate the baseline startle response of rats but did increase potentiation of startle by exposure to a bright light ("light-enhanced startle"), another measure of anxiety-like behavior (de Jongh, Groenink, van der Gugten, & Olivier, 2003; Walker & Davis, 1997a). Thus, withdrawal from chronic, continuous nicotine may potentiate responses to stress (e.g., exposure to a bright light), whereas withdrawal from discrete injections of nicotine may produce a more general increase in basal anxiety levels. It is also noteworthy that some studies of continuous nicotine infusion have waited 24 h after the cessation of infusion to examine changes in startle responding (Acri et al., 1995; Acri et al., 1991; Jonkman et al., 2008). In the present study, startle responding was elevated 2 h after the final dose of nicotine but had returned to baseline levels within 4 h of drug exposure. Assessments of withdrawal taken 24 h after nicotine may therefore miss the critical

window in which anxiety-like behavior can be detected in the absence of an explicit stressor.

The anxiogenic effect seen in our study is consistent with other measures of negative affect in animals going through nicotine withdrawal. In the elevated-plus maze, rats show a decrease in open arm time following repeated (Irvine et al., 2001) and continuous (Brioni et al., 1994) drug exposure. Additionally, intracranial self-stimulation (ICSS) thresholds are elevated during withdrawal from both continuously infused nicotine (Epping-Jordan et al., 1998) and from repeated nicotine injections (Bozarth, Pudiak, & KuoLee, 1998). Our results are also consistent with the 45-min half-life of nicotine in the rat (Matta et al., 2007). Withdrawal-potentiated startle was already apparent at 1 h and peaked at 2 h after nicotine administration, a time point at which the majority of the drug is metabolized and therefore displaced from receptors. Combined with the ability of nicotine replacement to reverse the withdrawal-potentiated startle effect, these data suggest that loss of nAChR occupancy is a key event in the production of nicotine withdrawal-potentiated startle.

Surprisingly, we did not find evidence of mecamylamine-precipitated withdrawal-potentiated startle (Experiment IV). This observation is inconsistent with the robust precipitated-withdrawal effects observed using other measures of nicotine withdrawal such as somatic signs (e.g., Malin et al., 1994), conditioned place aversion (e.g., Suzuki et al., 1996), or increases in brain-reward thresholds (e.g., Epping-Jordan et al., 1998), and with measures of naloxone-precipitated opiate withdrawal observed in our laboratory (A. C. Harris & Gewirtz, 2004; A. C. Harris et al., 2004). It seems

unlikely that the absence of an effect of mecamylamine was related to the dose used (1 mg/kg) because the same dose effectively precipitates withdrawal as assessed through ICSS thresholds (Watkins, Stinus, Koob, & Markou, 2000). This null effect is also unlikely to be related to differences in the properties of mecamylamine's constituent stereoisomers (Papke, Sanberg, & Shytle, 2001) since, in a preliminary investigation, we also have found no evidence of withdrawal-potentiated startle after injection of dihydro-β-erythroidine (DHβE), a selective α4β2 nAChR antagonist. Moreover, our results are supported by the recent study of the effects of withdrawal from chronic nicotine on light-enhanced startle, in which higher doses of both mecamylamine and DHβE were also found to be ineffective in precipitating withdrawal (Jonkman et al., 2008).

The fact that startle potentiation in both this and the Jonkman et al. (2008) study was seen during spontaneous but not precipitated withdrawal may suggest that the expression of nicotine withdrawal-potentiated startle is not dependent on decreased activity of nAChRs per se, but rather is the result of downstream mechanisms (perhaps involving norepinephrine release) that do not become activated during the time window examined in Experiment IV. In the spontaneous withdrawal procedure, loss of nAChR occupancy began well before the 2-h startle test; in contrast, competitive occupancy by mecamylamine began 5 min before the final startle test session. Allowing more time between mecamylamine injection and startle testing may therefore have been necessary to yield a positive result. Interestingly, the notion that different mechanisms may mediate spontaneous and precipitated nicotine withdrawal also gains support from a

recent finding that a corticotropin-releasing factor receptor antagonist blocked changes in ICSS thresholds induced by precipitated withdrawal only (Bruijnzeel, Zislis, Wilson, & Gold, 2007).

In addition to its implications regarding nicotine dependence, this study adds to the growing body of evidence that potentiated startle is a reliable measure of the negative affective consequences of withdrawal following repeated episodes of drug exposure. Intermittent exposure to opiates results in escalating levels of withdrawal-potentiated startle across repeated injections, similar to the results of the current study for intermittent nicotine exposure. Opiate withdrawal-potentiated startle is reduced by clonidine, and results from Experiment III of the current study indicate that this is also the case for nicotine withdrawal. The affective component of opiate withdrawal appears to be more severe, however, with potentiated startle present during the first withdrawal episode (A. C. Harris & Gewirtz, 2004) and increasing in severity with subsequent exposures (A. C. Harris et al., 2004). In contrast, in the current study, nicotine withdrawal-potentiated startle was only evident after multiple injections, and the number of injections required varied across experiments. The magnitude of opiate withdrawal is also frequently greater than that of nicotine withdrawal, measured as changes in ICSS thresholds (Kenny & Markou, 2005). These findings are consistent with reports that opiate withdrawal is more debilitating than tobacco withdrawal in humans (Hughes et al., 1994).

One of the advantages of using potentiated startle as a measure of withdrawal in animals is that the startle reflex is also a reliable, non-invasive measure of affective

changes in humans. Withdrawal-potentiated startle can be used to assess withdrawal states in human addicts and is therefore a promising model for translational research projects. Indeed, elevated startle magnitude has been observed in humans withdrawing from opiates (Stine et al., 2001) and ethanol (Krystal et al., 1997; Saladin, Drobes, Coffey, & Libet, 2002), and we are investigating whether similar effects can be observed during tobacco withdrawal (Engelmann & Cuthbert, 2008).

Perhaps the most important aspect of withdrawal-potentiated startle is that it allows changes in anxiety to be detected at the earliest stages of addiction. Symptoms of anxiety have been reported in human adolescents after only a few cigarettes (DiFranza et al., 2000), and the severity of such symptoms predicts the likelihood of future nicotine dependence (O'Loughlin et al., 2003). Studies that use the withdrawal-potentiated startle paradigm to identify brain mechanisms involved in the expression of negative affective symptoms of nicotine withdrawal may therefore be important in developing more effective treatments for tobacco dependence in humans, particularly in the vulnerable adolescent population.

CHAPTER 3: EMOTIONAL REACTIVITY TO EMOTIONAL AND SMOKING CUES DURING TOBACCO ABSTINENCE: POTENTIATED STARTLE AND P300 SUPPRESSION

Despite widespread awareness of the adverse health consequences of smoking and the benefits of quitting, it is estimated that 20 percent of the population continues to smoke cigarettes (Centers for Disease Control and Prevention, 2007). Most attempts to quit are unsuccessful, and in many cases relapse occurs during the first 2 days of abstinence (e.g., Gritz et al., 1991), which coincides with peak severity of withdrawal-induced affective changes such as increased anxiety and cigarette craving (e,g., Hughes, 1992, 2007). Smokers often report that anxiety, stress, or craving precipitate relapse (e.g., Allen et al., 2008; Piasecki et al., 1998; Piasecki et al., 2000; Shiffman et al., 1996) and that smoking a cigarette decreases anxiety (Juliano & Brandon, 2002; Kassel et al., 2007; Parrott, 1993, 1995; Shiffman, 1993). These effects provide support for negative reinforcement theories of tobacco addiction, which argue that dependent smokers continue smoking to escape or avoid negative affect (Baker et al., 2004; Watkins, Koob et al., 2000). Negative reinforcement theories predict that interventions that reduce the severity of aversive withdrawal symptoms or the anxiolytic consequences of smoking may be the most effective smoking cessation therapies, and assessment of smokers' emotional responses is becoming common in clinical investigations (e.g., R. West et al., 2008).

Studies of withdrawal-induced affective changes have relied almost exclusively on self-report measures. This introduces several threats to reliability and validity,

including variability in self-reported emotion and craving as a function of past withdrawal episodes (Tate, Pomerleau, & Pomerleau, 1993), smokers' expectancies about withdrawal symptoms (Gottlieb, Killen, Marlatt, & Taylor, 1987; Hughes, Pickens, Spring, & Keenan, 1985), and repeated administration of the same questionnaire (D. G. Gilbert et al., 1998). Many self-report measures may also lack sensitivity to subtle cognitive and emotional changes induced by nicotine administration (Tiffany, 1990). Furthermore, self-report measures cannot be used in animal models, increasing the difficulty of translating preclinical studies of potential smoking cessation treatments to clinical trials. It is therefore important to develop objective behavioral and physiological measures that can be used in conjunction with subjective measures (Lang et al., 1990; Lang, Bradley, & Cuthbert, 1998). This paper focuses on the use of emotion-modulated startle as an objective, psychophysiological measure of smoking-related emotional responses.

Emotion-modulated startle refers to changes in the amplitude of the acoustically elicited startle reflex as a function of the emotional valence of stimuli that are present when the startle probe is delivered: startle amplitude increases in the presence of aversive stimuli (fear-potentiated startle) and decreases in the presence of pleasant stimuli (pleasure-attenuated startle). Fear-potentiated and pleasure-attenuated startle have been observed reliably in humans (Grillon & Davis, 1997; Grillon et al., 1994; Schupp et al., 2004) and in nonhuman animals (Davis & Astrachan, 1978; Schmid et al., 1995). The basic neural circuitry of fear-potentiated startle has been identified (e.g., Walker et al., 2003) and suggests that the degree of startle potentiation or inhibition is

reflective of the relative activation of appetitive or defensive motivational systems in the brain (Lang et al., 1990; Lang, Bradley, & Cuthbert, 1998). The potential for detailed neurobiological analysis of affective startle modulation suggests that startle reflex measurement may be a useful model of affective changes induced by tobacco withdrawal.

Indeed, startle amplitude is potentiated in rats withdrawing from repeated daily nicotine injections (Engelmann, Radke, & Gewirtz, 2009) or continuously-infused nicotine (Helton et al., 1993), which is consistent with the emergence of an anxiety-like state during tobacco withdrawal. In human smokers there is no evidence of such potentiation of "baseline" startle (Della Casa, Hofer, Weiner, & Feldon, 1998; Duncan et al., 2001; Grillon, Avenevoli, Daurignac, & Merikangas, 2007; Hogle & Curtin, 2006; Mueller, Mucha, & Pauli, 1998), likely due to large individual differences in startle amplitude (cf. Blumenthal et al., 2005). In a number of studies, however, abstinent smokers have shown greater potentiated startle to emotive cues (Cinciripini et al., 2006; Grillon et al., 2007; Hogle & Curtin, 2006; but see Geier, Mucha, & Pauli, 2000; Piper & Curtin, 2006). This suggests that drug deprivation alters the response of brain motivational systems to the presentation of negative emotional cues.

In contrast to negative affective pictures, tobacco-related pictures tend to inhibit startle reactivity in abstinent and nonabstinent smokers (Cinciripini et al., 2006; Geier, Mucha, & Pauli, 2000; but see Orain-Pelissolo, Grillon, Perez-Diaz, & Jouvent, 2004). These results might suggest that tobacco cues are, in general, appetitive for smokers, even in states of deprivation. On the other hand, the cues may only have been

appetitive because the abstinent smokers were allowed to smoke immediately after the test session. Had the prospect of smoking a cigarette not been imminent, it is possible that smoking cues would have been aversive, eliciting responses associated with conditioned withdrawal (Poulos et al., 1981; Wikler, 1973) or "frustrative nonreward" (cf. Drobes et al., 2001; Hull, 1943). To resolve this issue, the current study examined whether smoking cues would potentiate startle when presented 24 hrs into a 48 hr period of abstinence.

This study also extended the cue reactivity literature by measuring the startle probe P3 in addition to startle blink amplitude. Schupp et al. (1997) demonstrated that the P300 (P3) event-related potential elicited by startle probes can be used as a measure of the attention-arousing characteristics of an emotional picture, with smaller P3 magnitude during highly arousing pleasant or unpleasant pictures than during low-arousal neutral pictures (also see Bradley, Codispoti, & Lang, 2006; Cuthbert, Schupp, Bradley, McManis, & Lang, 1998; Keil et al., 2007; Schupp et al., 2004). A similar phenomenon is seen in dual task procedures, where demanding foreground tasks result in decreased P3 magnitude to background stimuli (e.g., Isreal, Chesney, Wickens, & Donchin, 1980; Roth, Dorato, & Kopell, 1984). By measuring both startle blink amplitude and startle probe P3 suppression, it is possible to measure concurrently the effects of the emotional cue on relative activation of appetitive and defensive motivational systems and recruitment of cortical arousal systems (Schupp et al., 2004).

Previous research has found evidence of startle probe P3 suppression during tobacco pictures in nonabstinent smokers, consistent with the idea that tobacco-related

cues are motivationally significant to smokers (Versace et al., 2010). In the current study, we extended the measurement of startle probe P3 suppression to abstinent smokers. Because tobacco-related stimuli are thought to be more salient to abstinent smokers than nonabstinent smokers (e.g., Sayette & Hufford, 1994), we predicted that startle probe P3 suppression during tobacco pictures would be greater in abstinent smokers than in nonabstinent smokers. We also predicted that smokers reporting high levels of cigarette craving would have more startle probe P3 suppression during tobacco pictures than smokers reporting low levels of craving.

Method

Design

This study was designed to measure psychophysiological responses during an emotional picture viewing task 24 h into a 48-h period of tobacco abstinence. Participants came to the laboratory for three sessions (Figure 3-1). During Visit 1, all participants completed several baseline measures and smokers were randomly assigned to the abstinent group or nonabstinent group. Visits 2 and 3 were scheduled 24 h apart, within 3 weeks of Visit 1. Psychophysiological recording during the picture viewing task took place during Visit 2. Smokers assigned to the abstinent group were instructed to stop smoking 24 h before their scheduled time for Visit 2 through the end of Visit 3. Smokers assigned to the nonabstinent group were instructed to follow their normal smoking routine during the same 48-h period, and to smoke one additional cigarette at the start of Visits 2 and 3.

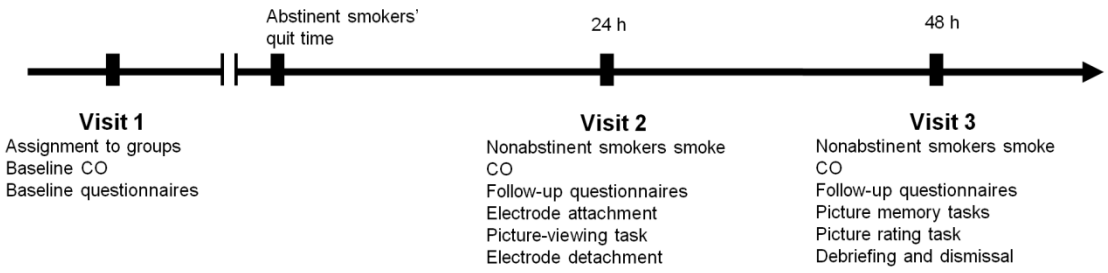

Figure 3-1. Timeline of laboratory visits. Visit 1 was an initial baseline and screening session. Visits 2 and 3 were scheduled 24 h apart, within 3 weeks of Visit 1. Smokers randomly assigned to the abstinent group were asked to stop smoking from 24 h before the start of Visit 2 through the end of Visit 3. Smokers randomly assigned to the nonabstinent condition continued their normal pattern of smoking throughout the study, and smoked one additional cigarette at the start of Visits 2 and 3.

Participants

Participants were recruited via advertisements posted throughout the university campus and the surrounding community, and via campus-based websites. These advertisements solicited the participation of nonsmokers and daily smokers between the ages of 18 and 35 who were not currently seeking treatment for smoking cessation. Exclusion criteria included current diagnosis of major psychiatric illness, medical conditions or use of medications that would influence psychophysiological recording, and current use of over the counter or prescription stop-smoking remedies or current participation in stop-smoking counseling. Participants had a choice of $50 or extra credit for their psychology course as compensation for completing all three visits. Smokers assigned to the abstinent group who met biochemical criteria for abstinence verification using expired air carbon monoxide (CO) received an additional cash bonus of $25 or additional course credit. Expired-air CO is a well-established, inexpensive, and noninvasive method of verifying short-term tobacco abstinence (Jarvis, Russell, & Saloojee, 1980; Jarvis, Tunstall-Pedoe, Feyerabend, Vesey, & Saloojee, 1987).

A total of 81 participants meeting these criteria gave written, informed consent to participate in the study: 24 nonsmokers (15 female), 28 smokers randomly assigned to the nonabstinent condition (14 female), and 29 smokers randomly assigned to the abstinent condition (16 female). The current analyses are limited to nonsmokers who reported never smoking a cigarette (n = 19, 11 female) and smokers with baseline expired-air carbon monoxide (CO) levels greater than 6 parts per million (ppm; n = 17 nonabstinent, 8 female and n = 18 abstinent, 9 female; e.g., Deveci, Deveci, Acik, & Ozan, 2004; Marrone, Paulpillai, Evans, Singleton, & Heishman, 2010)

All psychophysiological and picture-rating data from one female nonsmoker were lost due to equipment failure, two smokers (1 female) assigned to the abstinent condition dropped out of the study before the psychophysiological recording session, and one male smoker assigned to the abstinent condition failed to maintain abstinence for 24 hours prior to the psychophysiological recording session, leaving final sample sizes of 18 nonsmokers (10 female), 17 nonabstinent smokers (8 female) and 15 abstinent smokers (8 female). Data from 5 abstinent smokers (4 female) who were successfully abstinent at the start of Visit 2 but not at the start of Visit 3 were included in this analysis, as the primary interests of this study were the psychophysiological responses collected during Visit 2. The psychophysiological responses of this subgroup did not significantly differ from those of smokers who remained abstinent for the entire 48 hour period.

Stimuli

Sixty full-color, 1024 x 768 pixel stimuli were presented via a 41 x 31 cm LCD video monitor (Model 2001FP, Dell, Round Rock, TX), subtending a horizontal viewing angle of 26 degrees. These stimuli were classified according to four categories, with 15 pictures in each category: low-arousal neutral, high-arousal pleasant, high-arousal unpleasant, and tobacco. The pleasant, neutral, and unpleasant stimuli were selected from the International Affective Picture System, a set of pictures that have previously been rated on measures of affective valence, arousal, and dominance (Lang, Bradley, & Cuthbert, 1999). The specific IAPS pictures selected for this study were based on stimuli that have been found to be effective in previous studies in our laboratory (Cuthbert et al., 2007) and in the laboratories of others conducting similar studies (Drobes et al., 2001).[1]

Twelve of the tobacco pictures were obtained from a set of 48 collected by the authors from internet searches and print media (Engelmann & Cuthbert, 2008) and 3 were selected from a set generously provided by the lab of Paul Cinciripini (Carter et al., 2006). All tobacco pictures had been previously rated in our laboratory by smokers and nonsmokers on the same measures of affective valence, arousal, and dominance used to rate the IAPS pictures. The tobacco pictures used in this study were rated similarly to high-arousal pleasant IAPS pictures by smokers and similarly to low-arousal neutral IAPS pictures by nonsmokers (Engelmann & Cuthbert, 2008). These

[1] The IAPS pictures used were: Pleasant: 4606, 4611, 4651, 4653, 4687, 4694, 7330, 7410, 7450, 7470, 7488, 8034, 8117, 8499, 8502; Neutral: 2383, 2516, 2880, 2980, 5531, 5740, 7050, 7080, 7095, 7170, 7175, 7179, 7205, 7550, 7705; Unpleasant: 1051, 1270, 1300, 1930, 2095, 6022, 6200, 6243, 6244, 6260, 8480, 9042, 9290, 8342, 9592.

pictures contained scenes such as people preparing to smoke, people smoking alone or in social situations, and lit cigarettes with smoke clearly visible.[2]

Psychophysiological measures

The startle reflex was elicited using a 50-ms, 95-dB white noise burst with instantaneous rise time, generated by a Coulbourn (Whitehall, PA) V85-05 noise generator, amplified using a Radio Shack (Fort Worth, TX) 40-watt public address amplifier (Cat. No. 32-2054), and delivered binaurally via stereo headphones (Sennheiser PMX 100, Old Lyme, CT). Startle blink amplitude was measured using electromyography (EMG) with two 4-mm diameter Ag-AgCl electrodes (No. 701507-F, Rochester Electro-Medical, Tampa, FL) placed on the surface of the skin over the orbicularis oculi muscle according to the recommendations of Fridlund and Cacioppo (1986) and Blumenthal et al. (2005). Orbicularis EMG was amplified using a Coulbourn V75-05 bioamplifier, filtered with a bandpass of 28 - 500 Hz (Blumenthal et al., 2005) using a Coulbourn V75-48 high performance bandpass filter, and digitized at a sampling rate of 1000 Hz from 100 ms before startle probe onset through 250 ms after startle probe offset using an analog to digital converter (LabMaster DPCI, Scientific Solutions, Mentor, OH) and a computer running VPM software (Cook, 2003; Cook, Atkinson, & Lang, 1987).

The electroencephalogram was recorded using 4-mm diameter Ag-AgCl electrodes at the Fz, Cz, and Pz electrode sites of the International 10-20 System

[2] The tobacco stimuli from Carter et al. (2006) were: 402, 403, and 414. The remaining smoking stimuli were from Engelmann and Cuthbert (2008): S254, S359, S369, S377, S413, S435, S441, S454, S457, S460, S466, and S479. These stimuli are available upon request from the author.

(Jasper, 1958), referenced to linked mastoids (models E271-LS for scalp leads and E220N-LS for mastoid leads, In Vivo Metric, Healdsburg, CA). EEG traces were amplified and bandpass-filtered (0.1 - 40 Hz) using a Coulbourn (Whitehall, PA) V75-08 EEG amplifier, and digitized at a sampling rate of 125 Hz. For purposes of correcting eye movement artifacts in the EEG data, vertical electrooculogram (vEOG) was obtained by splitting the signal from the orbicularis EMG electrode placed directly below the pupil of the subject's non-dominant eye and another electrode placed directly above the pupil. The vEOG signal obtained was amplified and bandpass-filtered (0.1 - 40 Hz) using a Coulbourn V75-04 bioamplifier and digitized at a sampling rate of 125 Hz. An additional electrode placed on the forehead provided the signal ground for the EEG, EMG, and vEOG channels.

Procedure

Participants who expressed interest in the study and met requirements for participation (as determined by phone and email questionnaires) were invited to come to the laboratory for Visit 1. During this visit, each participant provided written informed consent, was further screened for the inclusion and exclusion criteria mentioned above, and was given an expired-air CO score (the average of two successive breath samples taken with a PiCo Smokerlyzer, Bedfont Scientific USA, Williamsburg, VA). All participants completed a series of questionnaires that addressed smoking history, trait fear (Fear Survey Schedule; FSS; Wolpe & Lang, 1964), personality traits (Emotionality, Activity, Sociability, and Impulsivity Inventory; EASI; Buss & Plomin, 1975, 1984), and state and trait anxiety (Spielberger State and Trait Anxiety Inventory;

STAI; Spielberger, 1979). Smokers completed additional questionnaires that assessed nicotine dependence (Fagerstrom Test of Nicotine Dependence; FTND; Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991), nicotine withdrawal symptoms experienced over the past 24 h (Minnesota Nicotine Withdrawal Scale; MNWS; Hughes & Hatsukami, 1998), and current cigarette craving (Questionnaire of Smoking Urges; QSU; Tiffany & Drobes, 1991). The QSU was scored using two factors. Factor 1 (QSU1) consisted of items related to the craving or urge for pleasurable consequences of cigarette smoking, and Factor 2 (QSU2) consisted of items related to an urge to smoke to avoid negative affect or aversive withdrawal symptoms (Tiffany & Drobes, 1991). After completing the questionnaires, smokers were informed of their random assignment to the abstinent or nonabstinent condition, and Visits 2 and 3 were scheduled.

Upon arrival for Visit 2, smokers assigned to the nonabstinent group were asked to completely smoke one of their regular cigarettes. All participants provided expired-air CO samples, and completed questionnaires about their smoking over the past 24 h. Abstinent smokers were classified as successfully abstinent if their CO level was less than or equal to 6 ppm or half of their baseline level (whichever was lower) and if they reported smoking no cigarettes over the past 24 h. These CO criteria have been shown to reliability detect tobacco abstinence (e.g., Marrone et al., 2010). All participants then completed the state scale of the STAI, and all smokers completed the MNWS and the QSU. Following the completion of questionnaires, several electrodes for psychophysiological recording were placed on the non-dominant hand, arms, face, and

scalp. The picture-viewing task began immediately after electrode attachment. This task consisted of a 3-min relaxation baseline, followed by three picture viewing blocks. At the start of each block, participants were instructed to pay attention to the pictures and that the sensors were going to measure their "physiological reactions to the pictures." The first block was a practice block, and consisted of four pictures (one from each category) and five startle probes. Data from the practice block were not analyzed. The second and third blocks each consisted of 30 pictures and 30 startle probes. On each trial, the picture was presented for 6 s, followed by an intertrial interval (ITI) ranging from 18-24 s, during which a gray screen was presented. Pictures were presented in a random order with respect to content category, and four different orders were used across subjects to reduce the influence of order effects on the results. During 12 of the 15 pictures from each category, a startle probe was presented 2.5 or 4.5 s after picture onset. Twelve additional startle probes were delivered during the ITIs (8, 10, or 12 s after picture offset). At the conclusion of the picture-viewing task, the electrodes were detached, and the participants completed a brief questionnaire and were dismissed until Visit 3.

The beginning of Visit 3 used the same procedure as the beginning of Visit 2 (i.e., nonabstinent smokers smoke, CO measurement, and questionnaires). All participants then completed two picture memory tasks (free recall and recognition), the results of which will not be reported here, followed-by a subjective ratings task. During the subjective ratings task, participants viewed each picture that was presented during the psychophysiology session. Immediately after viewing each picture for as long or

short a time as desired, participants used a computerized visual analog scale with five anchor points to rate the picture on the dimensions of affective valence and arousal (Hodes, Cook, & Lang, 1985). Participants were then debriefed, compensated, and dismissed.

Physiological data reduction

For each startle probe, orbicularis oculi EMG was extracted from 100 ms before probe onset through 250 ms after probe offset. The EMG traces were digitally rectified and low-pass filtered using an infinite impulse response filter that simulated a single-pole Butterworth RC integrative filter with a time constant of 125 ms (Cook, 2003; Cook & Miller, 1992; Drobes et al., 2001). The filtered data were hand-scored with the assistance of software that provided onset latency, peak latency, and blink magnitude in μV for each trial (Balaban, Losito, Simons, & Grahm, 1986; Cook, 2003). Trials with excessive electrical artifact, spontaneous blinks, and blinks with an onset latency of less than 20 ms or greater than 120 ms were rejected and not included in further analyses. Trials with no evidence of artifact but also no evidence of an eyeblink were assigned a startle magnitude of 0 μV. Participants with > 30% of startle trials rejected due to artifact were not included in further analyses. This criterion resulted in the exclusion of 1 nonabstinent smoker. Participants who did not blink on at least 60% of artifact-free trials were considered "non-blinkers" and were also excluded from further analyses. This criterion resulted in the exclusion of 4 nonsmokers, 2 nonabstinent smokers, and 3 abstinent smokers. Additionally, equipment failure resulted in the loss of all startle data

for 1 nonsmoker and 1 nonabstinent smoker. Thus, final sample sizes for startle data were 13 nonsmokers, 13 nonabstinent smokers, and 12 abstinent smokers.

To correct for individual differences in the overall size of startle blinks, blink size was quantified as an amplitude T-score with each subject serving as his/her own reference distribution (Blumenthal et al., 2005). After eliminating all trials rejected due to artifact and all trials with 0 μV blink magnitude, the remaining trials were converted to standardized T-scores ($M = 50$, $SD = 10$). The T-scores were then averaged across picture category, giving each subject a mean startle amplitude score for each picture category.

For each subject, raw EEG waveforms were digitally filtered using infinite impulse response filters with a high pass of 0.1 Hz and low pass of 40 Hz, followed by extraction of epochs from 300 ms before startle probe onset through 500 ms after startle probe offset. Epochs with off-scale or flat EEG or vEOG traces or with large (> 300 μV) deflections in the raw EEG were rejected, followed by eye movement correction using the algorithm of Gratton, Coles, and Donchin (1983) implemented in Fortran 77 (Miller, Gratton, & Yee, 1988). The epochs from individual trials were then averaged according to the category of picture that was on the screen when the startle probe was presented: tobacco, pleasant, neutral, or unpleasant. These average waveforms were baseline-corrected by subtracting the mean of the 120-ms window immediately preceding startle probe onset from all points in the waveform (Schupp et al., 1997). The P3 was quantified as the average value of the baseline-corrected waveform in a window lasting from 260-340 ms after startle probe onset (Keil et al., 2007). Equipment failure

resulted in the loss of startle probe P3 data for 2 nonsmokers and 1 nonabstinent smoker, resulting in final sample sizes for P3 data of 16 nonsmokers, 16 nonabstinent smokers, and 15 abstinent smokers.

Statistical analysis

Statistical analyses were conducted using SYSTAT 12 (SYSTAT Software, Chicago, IL). Participant characteristics and questionnaire scores that were only obtained during Visit 1 were analyzed using one-way ANOVA with group as a between-subjects factor, with significant results followed by comparison of individual group means using Tukey's Honestly Significant Difference (HSD). Expired-air CO and questionnaire measures that were obtained during all three laboratory visits were analyzed using group x session mixed model ANOVAs, with significant interactions followed by simple effects tests for day or group using one-way ANOVA (Maxwell & Delaney, 1990).

Psychophysiological responses to the pictures and SAM ratings were analyzed by computing difference scores for each subject's mean response to each category of motivationally significant picture (tobacco, pleasant, and unpleasant) from their mean response to neutral pictures (e.g., Versace et al., 2010). These difference scores were entered into group (nonsmoker, nonabstinent, abstinent) x picture category (tobacco, pleasant, unpleasant) ANOVAs. Significant group x picture category interactions were followed by tests for simple effects of group using between-subjects ANOVA followed by Tukey HSD *post hoc* tests. In addition to these analyses, an *a priori* one-way ANOVA was used to test for significant between-groups differences in the [unpleasant

– neutral] difference scores for startle amplitude as a measure of fear-potentiated startle (Vaidyanathan, Patrick, & Bernat, 2009). In all cases, the significance criterion was set at $p < .05$ and multivariate tests (Wilks λ and its approximate F statistic) were used for effects involving repeated measures (Johnson & Wichern, 2002).

To assess the relationship between physiological responses to the pictures and self-reported cigarette craving or anxiety, we assigned participants to low vs. high scoring groups for QSU1, QSU2, MNWS and STAI-State based on median splits of the change in questionnaire scores from Visit 1 to Visit 2, and STAI-Trait based on median splits of scores obtained during Visit 1. High vs. low subgroup assignment based on the median split was used as a between-subjects factor in smoking group x questionnaire subgroup x picture category ANOVAs, and significant main effects or interactions involving questionnaire scores were followed up with exploratory correlational analyses of the relationship between questionnaire scores and physiological responses.

Results

Participant demographics and smoking history

Participants were adults in their early twenties, and smokers were considered light to moderate smokers according to the number of cigarettes smoked per day, CO levels produced during Visit 1, and scores on the FTND and HSI (Table 3-1). Nonabstinent smokers and abstinent smokers did not differ significantly on the number of cigarettes smoked per day, maximum daily smoking rate, number of years they have been smoking, number of serious quit attempts, longest duration of abstinence during a quit attempt, FTND score, and HSI score (see Table 3-1 for group F tests).

Table 3-1. Participant demographics and smoking history (Mean ± SEM)

	Nonsmokers	Nonabstinent Smokers	Abstinent Smokers	Group ANOVA
N (female)	18 (10)	17 (8)	15 (8)	
Percent minority	33.3	29.4	26.7	
Age (years)	20.8 ± 0.7	22.2 ± 1.2	21.7 ± 0.6	$F < 1$
Current CPD		10.9 ± 1.6	11.0 ± 1.5	$F < 1$
Maximum CPD		18.2 ± 3.2	15.0 ± 1.6	$F < 1$
Years smoked		7.3 ± 1.4	5.2 ± 0.7	$F = 1.57$
Quit attempts		4.0 ± 1.4	2.8 ± 1.3	$F < 1$
Maximum days abstinent		104.8 ± 54.5	98.4 ± 71.4	$F < 1$
FTND score		2.9 ± 0.4	3.3 ± 0.4	$F < 1$
HSI score		1.9 ± 0.3	2.1 ± 0.3	$F < 1$
Session 1 CO	2.4 ± 0.3^{a}	12.6 ± 1.5^{b}	13.5 ± 1.2^{b}	$F = 32.65$***
Session 2 CO	2.0 ± 0.2^{a}	13.6 ± 1.8^{b}	2.4 ± 0.3^{a}	$F = 37.61$***
Session 3 CO	1.8 ± 0.1^{a}	13.6 ± 2.2^{b}	4.5 ± 1.1^{a}	$F = 20.20$***

Note: CPD = Cigarettes per day. The FTND was scored on a scale of 1-10, the HSI was scored on a scale of 1-6. *Asterisks* indicate statistically significant *F* tests in a one-way ANOVA for group (*** $p < .001$). For ANOVAs of measures taken from all three groups, all *F*s had 2 numerator and 47 denominator degrees of freedom. On measures for which the ANOVA was statistically significant, means that do not share superscripts significantly differed in Tukey *post hoc* tests. For group ANOVAs involving measures taken only in smokers, *F*s had 1 numerator and 30 denominator degrees of freedom, with two exceptions, due to some participants who never attempted to quit and others who could not recall for how long they had been abstinent during previous quit attempts: Number of quit attempts had *n*s of 16 nonabstinent smokers and 13 abstinent smokers, which lead to $F(1,27)$ and maximum days abstinent had *n*s of 13 nonabstinent smokers and 10 abstinent smokers, which lead to $F(1,21)$.

Abstinence verification

Examination of expired-air CO readings revealed a pattern of results that was consistent with smoking throughout the day before all three laboratory sessions in smokers assigned to the nonabstinent condition, and with abstinence prior to Visits 2 and 3 in smokers assigned to the abstinent condition. Group x session ANOVA revealed a significant group x visit interaction [$F(4,92) = 13.8$, $p < .001$, $\lambda = .39$], driven by main effects of group during each visit (Table 3-1). As expected, both groups of smokers produced significantly greater CO levels than nonsmokers during Visit 1, but only nonabstinent smokers produced significantly greater CO levels than nonsmokers during Visits 2 and 3. For all participants, self-reported abstinence agreed with abstinence classification based on CO levels.

Self-report measures

When examining the results of questionnaires used to measure trait characteristics (FSS, EASI, and the STAI-Trait), there were significant effects of group on two measures: The impulsivity subscale of the EASI and the trait anxiety scale of the STAI (Table 3-2). All three groups differed significantly on impulsivity scores, with abstinent smokers scoring the highest, followed by nonabstinent smokers and nonsmokers. For trait anxiety, abstinent smokers scored significantly higher than nonsmokers, but neither group scored significantly different from nonabstinent smokers.

Scores on questionnaires used to measure state characteristics (STAI-State, MNWS, and QSU) indicated increased negative affect and cigarette craving during tobacco abstinence. There were statistically significant group x session interactions for MNWS withdrawal symptom scores [$F(2,29) = 3.91$, $p < .05$, $\lambda = .79$], QSU1 scores [$F(2,29) = 7.35$, $p < .01$, $\lambda = .66$], and QSU2 scores [$F(2,29) = 8.58$, $p < .01$, $\lambda = .63$], and the group x session interaction approached statistical significance for state anxiety scores [$F(4,92) = 2.30$, $p = .07$, $\lambda = .83$]. These interactions were the result of abstinent smokers scoring higher than nonabstinent smokers on the MNWS and QSU during Visits 2 and 3, but not during Visit 1, and abstinent smokers scoring higher on the STAI-State scale than both nonsmokers and nonabstinent smokers during Visit 2 and higher than nonsmokers during Visit 3 (Table 3-2).

Table 3-2. Self-report measures of personality traits, anxiety, withdrawal symptoms, and craving (Mean ± SEM)

	Session	Nonsmokers	Nonabstinent Smokers	Abstinent Smokers	Group ANOVA
FSS	1	2.0 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	F < 1
EASI-Sociability	1	3.7 ± 0.2	3.6 ± 0.2	3.5 ± 0.2	F < 1
EASI-Activity	1	2.9 ± 0.2	2.9 ± 0.2	2.8 ± 0.1	F < 1
EASI-Impulsivity	1	2.4 ± 0.1[a]	3.0 ± 0.1[b]	3.4 ± 0.1[c]	F = 16.00***
EASI-Fear	1	2.2 ± 0.1	2.5 ± 0.1	2.5 ± 0.2	F = 1.41
EASI-Anxiety	1	2.0 ± 0.1	2.3 ± 0.2	2.5 ± 0.2	F = 1.54
STAI-Trait	1	1.8 ± 0.1[a]	2.0 ± 0.1[a,b]	2.3 ± 0.1[b]	F = 6.21**
STAI-State	1	1.6 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	F = 2.59
	2	1.7 ± 0.1[a]	1.9 ± 0.2[a]	2.4 ± 0.1[b]	F = 8.34**
	3	1.5 ± 0.1[a]	1.9 ± 0.2[a,b]	2.2 ± 0.2[b]	F = 8.16**
MNWS	1		1.4 ± 0.2	1.4 ± 0.2	F < 1
	2		1.3 ± 0.2	2.0 ± 0.2	F = 9.02**
	3		1.0 ± 0.2	1.9 ± 0.2	F = 8.84**
QSU Factor 1	1		4.8 ± 0.3	4.4 ± 0.3	F < 1
	2		3.8 ± 0.3	5.5 ± 0.3	F = 14.14**
	3		4.0 ± 0.3	5.5 ± 0.4	F = 9.35**
QSU Factor 2	1		2.9 ± 0.2	3.2 ± 0.4	F < 1
	2		2.5 ± 0.2	4.5 ± 0.4	F = 20.98***
	3		2.6 ± 0.3	4.1 ± 0.5	F = 8.07**

Note: The FSS and all scales of the EASI were measured on a 1-5 scale, both scales of the STAI were measured on a 1-4 scale, the MNWS was measured on a 0-4 scale, and both scales of the QSU were measured on a 1-7 scale. *Asterisks* indicate statistically significant *F* tests in a one-way ANOVA for group (**$p < .01$, ***$p < .001$). For ANOVAs of measures taken from all three groups, all *F*s had 2 numerator and 47 denominator degrees of freedom. On measures for which the ANOVA was statistically significant, means that do not share superscripts significantly differed in Tukey *post hoc* tests. For group ANOVAs involving measures taken only in smokers, all *F*s had 1 numerator and 30 denominator degrees of freedom. *n*s = 18 nonsmokers, 17 nonabstinent smokers, and 15 abstinent smokers.

Subjective ratings of the pictures

Mean valence and arousal ratings of the pictures are shown in Figure 3-2. Although the groups did not differ significantly in their arousal ratings of neutral pictures [$F(2,47) = 2.32$, $p > .1$], abstinent smokers rated neutral pictures as less pleasant than nonabstinent smokers [$F(2,47) = 4.65$, $p < .05$]. Analysis of difference scores between emotional and neutral pictures found a significant group x picture category interaction for both valence [$F(4,92) = 7.61$, $p < .001$, $\lambda = .57$] and arousal

[$F(4,92) = 4.71$, $p < .01$, $\lambda = .69$]. For both measures, simple effects tests found significant group effects on difference scores for tobacco pictures [valence: $F(2,47) = 20.62$, $p < .001$; arousal: $F(2,47) = 3.35$, $p < .05$], but not for pleasant or unpleasant pictures [all Fs$(2,47) < 1.72$, all ps $> .1$]. Tukey post-hoc tests revealed that both groups of smokers rated tobacco pictures as more pleasant, compared to nonsmokers. For arousal, abstinent smokers' ratings were higher than nonsmokers' ratings.

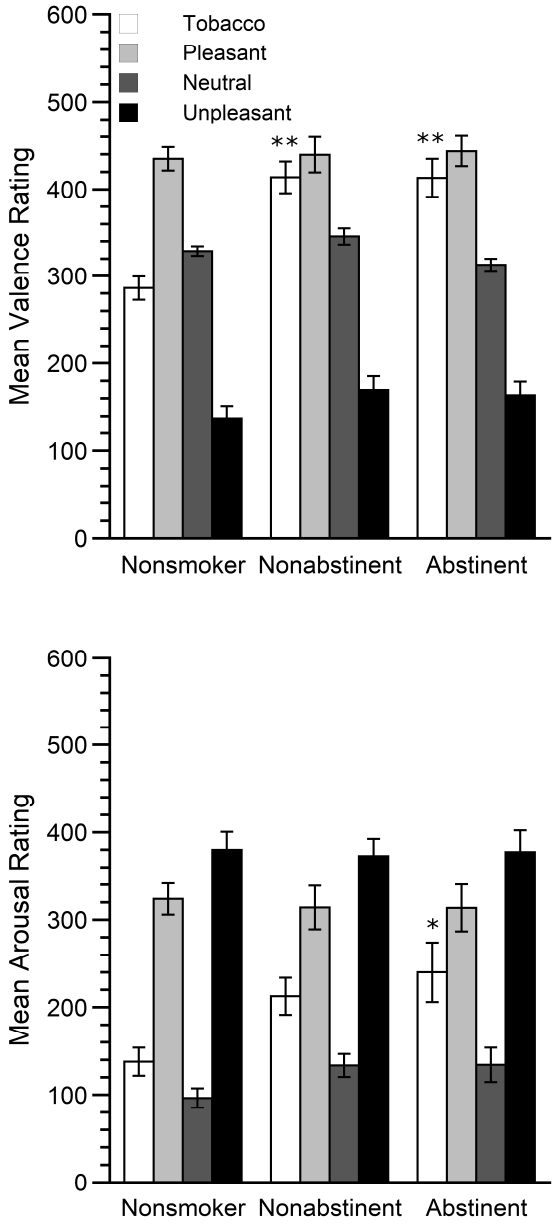

Figure 3-2. Valence (*upper panel*) and arousal (*lower panel*) ratings of the pictures from each category during the subjective ratings task. The ratings were obtained via a computer using a visual analog scale, and the values on the *y*-axis represent the mean coordinate along that scale. *Error bars* depict the mean ± 1 SEM. *n*s = 18, 17, and 15 for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively. * ($p < .05$) and ** ($p < .01$) indicate that the mean [emotional – neutral] difference score for the marked group and picture category was significantly different from the same score in the nonsmoker group, as determined by Tukey HSD *post hoc* tests.

Startle blink amplitude

Preliminary analyses found that raw startle amplitude (measured in μV) to probes delivered during the intertrial intervals or neutral pictures did not significantly differ between groups [*F*s(2,35) < 1.08, *p*s > .1]. This is consistent with previous research that has found no evidence of withdrawal-potentiated startle in the absence of emotional stimuli (e.g., Mueller et al., 1998). Startle amplitude T-scores are presented in Figure 3-3. Analysis of the difference in T-scores between emotional and neutral pictures found no significant group x picture category interaction [$F(4,68) < 1$, $p > .1$]. The main effect of picture category was significant [$F(2,34) = 8.42$, $p < .01$], which was the result of greater difference scores for unpleasant pictures than for tobacco or pleasant pictures. Although the group x picture category interaction was not significant, the *a priori* ANOVA for group differences in fear-potentiated startle (i.e., [unpleasant – neutral] difference scores) was statistically significant [$F(2,35) = 3.45$, $p < .05$]. The significant effect of group was the result of greater fear-potentiated startle in nonsmokers ($M = 4.57$, $SEM = 1.29$) and abstinent smokers ($M = 3.91$, $SEM = 1.46$) than in nonabstinent smokers ($M = -0.13$, $SEM = 1.41$). Tukey post-hoc tests indicated that the difference between nonsmokers and nonabstinent smokers was statistically significant ($p = .05$) and that the difference between nonabstinent and abstinent smokers approached significance ($p = .1$). There were no significant effects of group on [pleasant – neutral] [$F(2,35) = 2.04$, $p > .1$] or [tobacco – neutral] difference scores [$F(2,35) < 1$, $p > .1$].

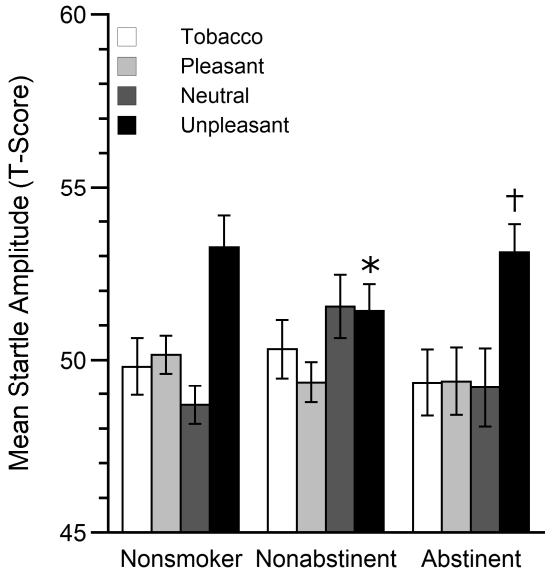

Figure 3-3. Mean blink amplitude during tobacco, pleasant, neutral and unpleasant pictures in nonsmokers, nonabstinent smokers, and abstinent smokers. The heights of the columns depict the mean standardized startle amplitude to auditory startle probes delivered during each picture category. *Error bars* depict the mean ± 1 *SEM*. *n*s = 13, 13, and 12 for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively. Symbols indicate the significance of the difference between the mean [emotional – neutral] difference score for the marked group and picture category and the same score in the nonsmoker (*, $p < .05$) or the nonabstinent group (†, $p < .1$), as determined by Tukey HSD *post hoc* tests.

Startle probe P3

Inspection of grand average waveforms (Figure 3-4) revealed a large P3 to the startle probe that was most pronounced at Pz, consistent with findings from other studies measuring the startle probe P3 (e.g., Cuthbert et al., 1998; Keil et al., 2007; Schupp et al., 1997; Schupp et al., 2004). Quantitative analysis of P3 magnitude was conducted on the single-subject ERP waveforms in a window lasting from 260-340 ms after startle probe onset (Keil et al., 2007), which are shown in Figure 3-5. There were no significant effects of group on P3 magnitude to neutral pictures at any electrode site [all Fs(2,44) < 1.34, all ps > .1]. Analysis of difference scores between emotional and neutral pictures found a significant group x picture category interaction at Pz [$F(4,86) = 3.56$, $p < .05$, $\lambda = .74$]. The interaction was the result of a significant simple effect of

group for unpleasant pictures [$F(2,44) = 6.95$, $p < .01$], but not for tobacco [$F(2,44) = 1.19$, $p > .1$] or pleasant [$F(2,44) < 1$, $p > .1$] pictures. Tukey post-hoc tests indicated that abstinent smokers had greater startle probe P3 suppression during unpleasant pictures than both nonabstinent smokers and nonsmokers.

At Fz, the group x picture category interaction was not statistically significant [$F(4,86) = 1.37$, $p > .1$, $\lambda = .88$] and this interaction only approached significance at Cz [$F(4,86) = 2.24$, p .07, $\lambda = .82$]. There were significant main effects of picture category at both electrode sites [Fz: $F(2,43) = 4.04$, $p < .05$, $\lambda = .84$; Cz: $F(2,43) = 4.68$, $p < .05$, $\lambda = .82$]. These main effects were the result of greater P3 suppression during tobacco and pleasant pictures (relative to neutral pictures) than during unpleasant pictures. Although the group x stimulus category interactions were not statistically significant, it is noteworthy there was greater P3 suppression to unpleasant pictures in abstinent smokers than in nonsmokers or nonabstinent smokers at both Fz and Cz, which is consistent with the results at Pz. In fact, the simple effect of group on P3 suppression during unpleasant pictures was statistically significant at Cz [$F(2,44) = 6.05$, $p < .01$] and approached significance at Fz [$F(2,44) = 2.60$, $p = .09$], but was not significant for tobacco or pleasant pictures at either electrode site [all Fs$(2,44) < 1$, all ps $> .1$].

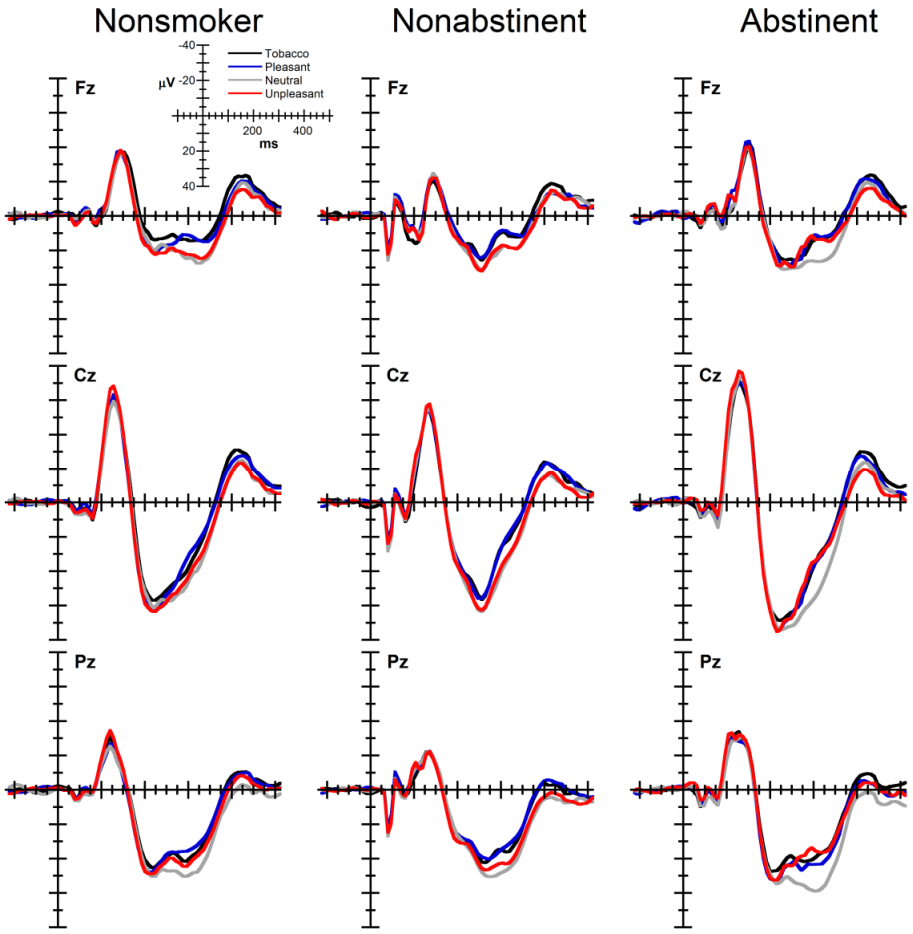

Figure 3-4. Grand average waveforms for event-related potentials to startle probes delivered during tobacco, pleasant, neutral, and unpleasant pictures. The data plotted range from 120 ms before through 500 ms after startle probe onset. Startle probe onset occurred at the 0-ms time point and all waveforms were baseline corrected using the 120-ms pre-stimulus baseline. The EEG was measured in μV, where negative values are plotted above the *x*-axis and positive values are plotted below the *x*-axis. Electrode sites (Fz, Cz, and Pz) are plotted in *rows* and groups (nonsmokers, nonabstinent smokers, and abstinent smokers) are plotted in *columns*. *n*s = 16, 16, and 15 for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively.

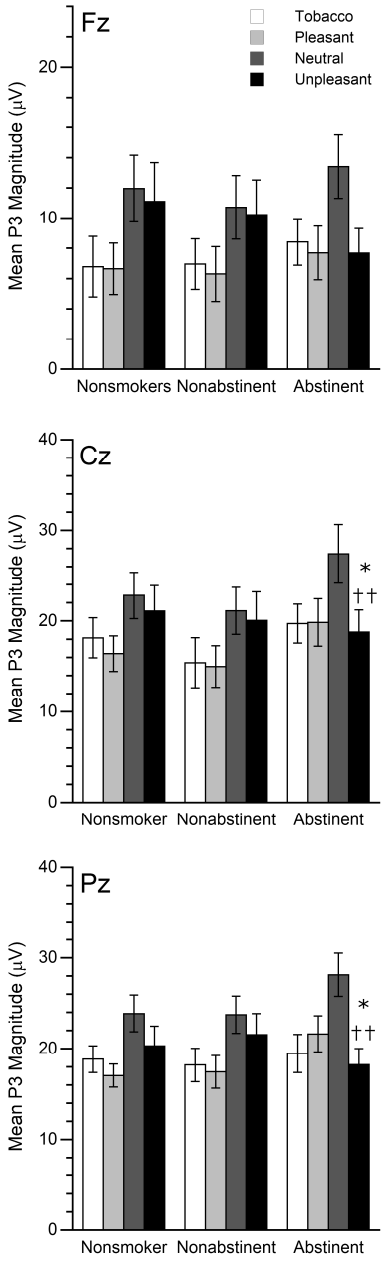

Figure 3-5. Mean startle-probe P3 magnitude during tobacco, pleasant, neutral and unpleasant pictures at the Fz (*upper panel*), Cz (*middle panel*), and Pz (*lower panel*) electrode sites in nonsmokers, nonabstinent smokers, and abstinent smokers. P3 magnitude was quantified for each picture category for each subject using a window average lasting from 260-340 ms after startle probe onset. The heights of the columns depict mean P3 magnitude to startle probes delivered during each picture category. *Error bars* depict the mean ± 1 *SEM*. *n*s = 16, 16, and 15 for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively. Symbols indicate the significance of the difference between the mean [emotional – neutral] difference score for the marked group and picture category and the same score in the nonsmoker (*, $p < .05$) or nonabstinent group (††, $p < .01$), as determined by Tukey HSD *post hoc* tests.

Self-reported craving and psychophysiology

Smokers in the nonabstinent and abstinent groups were assigned to "low" vs. "high" craving subgroups based on median splits of their change scores between Visits 1 and 2 for QSU1 and QSU2. The QSU1 scores in each of these subgroups are presented in the upper panel of Figure 3-6. Abstinence x QSU1 x picture category ANOVA found significant effects involving QSU1 scores at Fz, but not at Cz or Pz [QSU1 x picture category: $F(2,26) = 5.04$, $p < .05$, $\lambda = .72$]. Follow-up abstinence x QSU1 between-subject ANOVAs found a significant abstinence x QSU1 interaction for P3 suppression during tobacco pictures [$F(1,27) = 5.69$, $p < .05$], but not for P3 suppression during pleasant or unpleasant pictures [Fs$(1,27) < 1.57$, ps $> .1$]. [Tobacco – neutral] difference scores for startle probe P3 magnitude as a function of abstinence group and QSU1 subgroup assignment are plotted in the middle panel of Figure 3-6. The plot shows that the interaction was the result of a significant effect of QSU1 subgroup assignment in the abstinent smokers [$F(1,13) = 5.37$, $p < .05$], but not in the nonabstinent smokers [$F(1,14) < 1$, $p > .1$], with abstinent smokers who showed the largest increase in QSU1 scores from Visit 1 to Visit 2 also showing the greatest suppression of P3 magnitude to probes delivered during tobacco pictures.

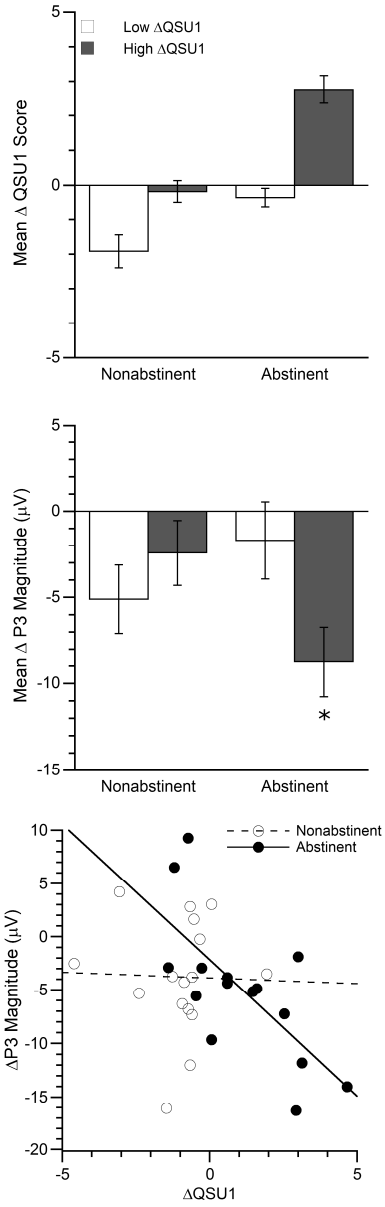

Figure 3-6. Effect of change in self-reported cigarette craving on startle probe P3 magnitude during tobacco pictures. *Upper panel*: Mean change in QSU1 scores from Visit 1 to Visit 2 (ΔQSU1) as a function of abstinence condition and median split on ΔQSU1 scores. *Middle panel*: Mean [tobacco – neutral] difference in startle probe P3 magnitude (ΔP3) at the Fz electrode site as a function of abstinence condition and median split on ΔQSU1 scores. *White columns* depict the mean ΔP3 magnitude of smokers falling below the median ΔQSU1score (*n*s = 8 and 8 for nonabstinent and abstinent smokers, respectively) and *gray columns* depict the mean ΔP3 magnitude for smokers falling above the median ΔQSU1 score (*n*s = 8 and 7 for nonabstinent and abstinent smokers, respectively). *Error bars* depict the mean ± 1 *SEM*. * Indicates a significant difference from the Abstinent-Low ΔQSU1 group ($p < .05$). *Lower panel:* Scatter plot and least squares regression lines for ΔQSU1scores (*x*-axis) and ΔP3 scores (*y*-axis) for nonabstinent smokers (*open circles* and *dashed line*) and abstinent smokers (*closed circles* and *solid line*).

To further investigate the nature of the significant group x QSU1 score interactions on P3 suppression during tobacco pictures, we computed Pearson product-moment correlations of the change in QSU1 scores from Visit 1 to Visit 2 and the [tobacco – neutral] difference scores for P3 magnitude. This correlation was statistically significant at Fz (r = -.69, p < .01; Figure 3-6, lower panel) and Cz (r = -.64, p = .01) and approached significance at Pz (r = -.50, p = .06) in abstinent smokers, but not in nonabstinent smokers (all |r|s < .17, all ps > .1). When computed for both nonabstinent and abstinent smokers as a single group, the correlation was significant at Fz (r = .41, p < .05) and approached significance at Cz (r = .31, p = .09) and Pz (r = .31, p = .09). Collectively, the significant interactions involving median splits on QSU1 scores and significant negative correlations between QSU1 scores and [tobacco – neutral] P3 scores suggest that smokers reporting the greatest abstinence-induced increases in cigarette craving had the greatest amount of startle-probe P3 suppression during the tobacco pictures.

We also examined whether subgroup assignments based on median splits on craving scores had an effect on the results of the emotion-modulated startle analysis. No main effects or interactions involving median split-based subgroup assignment for the QSU1 or QSU2 approached significance [Fs < 2.13, ps > .1]. The correlations between change in QSU1 or QSU2 scores and fear-potentiated startle also did not approach statistical significance when computed for all smokers (|r|s < .13, ps > .1) or for abstinent (|r|s < .45, ps > .1) and nonabstinent (|r|s < .18, ps > .1) smokers separately.

Self-reported anxiety and psychophysiology

Because there were differences between groups in trait anxiety measured during Visit 1, further analyses were conducted to see if the differences between nonabstinent and abstinent smokers in fear-potentiated startle and P3 suppression during unpleasant pictures were influenced by trait anxiety scores. We computed correlations between questionnaire scores and both fear-potentiated startle and [unpleasant – neutral] differences in P3 magnitude, similar to the analyses for [tobacco – neutral] P3 difference scores reported above. As shown in Figure 3-7, trait anxiety in abstinent smokers correlated negatively both with P3 difference scores at Fz during unpleasant pictures (r = -.62, p < .05) and with fear-potentiated startle (r = -.61, p < .05). These correlations were not significant in nonabstinent smokers (P3: r = -.15, p > .1; Startle: r = .04, p > .1) or nonsmokers (P3: r = .19, p > .1; Startle: r = -.18, p > .1). The correlations between trait anxiety and fear-potentiated startle were also not statistically significant when computed for abstinent and nonabstinent smokers as a single group (r = -.12, p > .1, n = 25) or for nonsmokers, nonabstinent smokers, and abstinent smokers as a single group (r = -.22, p > .1, n = 38). However, correlations between trait anxiety and P3 suppression remained significant when computed for nonabstinent and abstinent smokers as a single group (r = -.44, p < .05, n = 31) and for nonsmokers, nonabstinent smokers, and abstinent smokers as a single group (r = -.33, p < .05, n = 47).

Significant correlations between trait anxiety scores and physiological responses were limited to unpleasant pictures; correlations between [pleasant – neutral] or [tobacco – neutral] difference scores for P3 suppression or startle amplitude did not

approach statistical significance (all |*r*|s < .37, all *p*s > .1). No correlations involving other electrode sites or questionnaire measures approached statistical significance (all |*r*|s < .44, all *p*s > .1). This pattern of correlations suggests that abstinent smokers who reported greater trait anxiety during the baseline visit had greater startle probe P3 suppression but less fear-potentiated startle during unpleasant pictures than those who reported less trait anxiety.

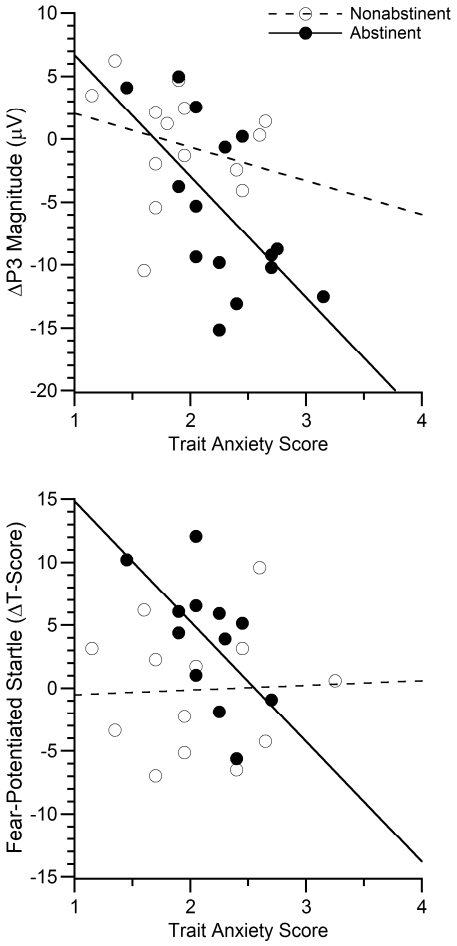

Figure 3-7. Relationship between self-reported trait anxiety and psychophysiological responses to unpleasant pictures. Scatter plot and least squares regression lines for scores on the trait scale of the STAI (*x*-axis) and [unpleasant – neutral] difference scores for startle probe P3 magnitude (upper panel, *y*-axis) and fear potentiated startle (lower panel, *y*-axis) for nonabstinent smokers (*open circles* and *dashed lines*) and abstinent smokers (*closed circles* and *solid lines*).

Discussion

This study examined startle blink amplitude and startle probe P3 suppression while nonsmokers, nonabstinent smokers, or smokers who had been abstinent for the past 24 h viewed pictures from four categories: tobacco, pleasant, neutral and unpleasant. Three patterns of results were evident. First, abstinent smokers appeared to be more emotionally reactive to unpleasant pictures than nonabstinent smokers, as indicated by greater fear-potentiated startle and P3 suppression. Second, abstinent smokers who reported the largest increases in cigarette craving during the first 24 h of abstinence showed greater P3 suppression during pictures of smoking-related cues than the other smokers in the study. Third, psychophysiological responses to unpleasant pictures were correlated with trait anxiety scores in abstinent smokers.

Tobacco abstinence increases emotional cue reactivity

Fear-potentiated startle is thought to reflect increased activation of defensive motivational systems in the brain (Lang et al., 1990; Lang, Bradley, & Cuthbert, 1998). In this study, nonsmokers and abstinent smokers showed robust fear-potentiated startle during unpleasant pictures, while nonabstinent smokers did not, suggesting that defensive motivational systems were less active in nonabstinent smokers than in nonsmokers or abstinent smokers. This effect is more likely due to the acute effects of nicotine intake than to the effects of nicotine withdrawal, because the nonabstinent smokers – whose most recent cigarette was within 30 minutes of the start of the picture viewing task – differed from both 24-h-deprived smokers and from nonsmokers, and because the nonsmokers and 24-h-deprived smokers did not differ in fear-potentiated

startle. This result is consistent with the findings of another study in which intranasal nicotine administration after 12 h of smoking abstinence reduced fear-potentiated startle (Cinciripini et al., 2006) and corrugator EMG (J. D. Robinson, Cinciripini, Carter, Lam, & Wetter, 2007) during unpleasant pictures and with subjective reports that smoking has an immediate anxiolytic effect (Parrott, 1993, 1995). Nicotine also has anxiolytic effects in animal models (Picciotto, Brunzell, & Caldarone, 2002), including potentiated startle paradigms (Engelmann et al., 2009; Vale & Green, 1996). Thus, although not all studies have found differences in fear-potentiated startle between nonabstinent and abstinent smokers (Geier et al., 2000; Piper & Curtin, 2006), there is a growing body of evidence in both the human and animal literature that nicotine administration reduces, and that withdrawal increases, fear-potentiated startle, as predicted by negative reinforcement theories of nicotine addiction (Baker et al., 2004).

Although a number of previous studies have assessed the effects of smoking and smoking cessation on startle reactivity, this study is the first, to our knowledge, to also assess the effects of smoking cessation on startle probe P3 suppression. The main finding in this data set was that tobacco abstinence markedly increased startle probe P3 suppression during unpleasant pictures, but not during pleasant pictures. This finding coheres with the results of other studies of smokers' attention to emotional pictures. For example, transdermal nicotine administration to 12-h abstinent smokers decreased P3 suppression to target stimuli in the presence of unpleasant distractors in a rapid visual information processing (RVIP) task (D. G. Gilbert et al., 2007), improved performance in the presence of unpleasant distractors in a similar RVIP task (D. G.

Gilbert et al., 2005), decreased interference caused by unpleasant words in an emotional Stroop task (Rzetelny et al., 2008), and reduced eye-gaze at negative pictures in a two-choice viewing task (D. G. Gilbert et al., 2008). Thus, there is considerable evidence that tobacco abstinence increases, and nicotine replacement decreases, selective attention to unpleasant stimuli. This, again, is supportive of negative reinforcement models of tobacco addiction (Baker et al., 2004).

Considered together, the relatively low level of startle potentiation during unpleasant stimuli in nonabstinent smokers and the relatively high level of startle probe P3 suppression during the same stimuli in abstinent smokers suggest that abstinent smokers were more emotionally reactive than nonabstinent smokers to unpleasant pictures. Interestingly, while the fear-potentiated startle effect was attributed to the acute effects of smoking, the startle probe P3 effect appeared to be due to the effects of abstinence; fear-potentiated startle did not differ between nonsmokers and abstinent smokers, whereas P3 suppression did not differ between nonsmokers and nonabstinent smokers. This difference between psychophysiological measures suggests that different aspects of smoking behavior (i.e., reduction of negative affect via smoking versus increased negative affect during withdrawal) may be associated with different brain motivational systems (Watkins, Koob et al., 2000).

Craving modulates emotional responses to tobacco pictures

Contrary to our predictions, the stronger emotional reactivity of the abstinent group did not, as a whole, extend to their responses to tobacco-related pictures. A significant effect emerged, however, when the level of cigarette craving was included in

the analysis. Specifically, those abstinent smokers who reported increases in cigarette craving (measured using the QSU1) between Visits 1 and 2 showed greater P3 suppression to the smoking-related cues. In fact, the Abstinent-High QSU1 group was the only group to show increased craving across sessions (Fig. 6, *upper panel*), suggesting that an overall effect of group on P3 suppression during tobacco pictures may have been eliminated by smokers whose craving levels did not rise during abstinence. Interestingly, the differences in psychophysiological responses during unpleasant pictures were not dependent on QSU scores. This suggests that P3 suppression to unpleasant pictures and tobacco pictures may be under the control of different aspects of tobacco deprivation, and underscores the importance of including multiple psychophysiological measures to multiple categories of emotional stimuli in cue reactivity studies.

Trait anxiety modulates emotional responses to unpleasant pictures

Not only were there group-level differences in fear-potentiated startle and startle probe P3 suppression during unpleasant pictures, but each of these measures was also related to individual differences in trait anxiety. Across nonsmokers, nonabstinent smokers, and abstinent smokers, higher trait anxiety scores were associated with greater P3 suppression during unpleasant pictures. Although previous research has not addressed this relationship, there is evidence using visual ERPs that more attentional resources are allocated to the processing of unpleasant stimuli in high trait-anxious vs. low-trait anxious individuals (Weinstein, 1995).

This raises the question of whether trait anxiety mediates the effect of higher P3 suppression seen in abstinent smokers. A closer examination of the patterns of differences between the three groups in trait anxiety and P3 suppression suggests this not to be the case. Although the abstinent smokers presented higher trait anxiety than the nonsmokers, neither of these groups differed significantly from nonabstinent smokers. In contrast, the abstinent smokers significantly differed from both the nonabstinent smokers and nonsmokers on P3 suppression during unpleasant pictures. Moreover, the negative correlation between trait anxiety scores and P3 suppression during unpleasant pictures was larger in abstinent smokers (r = -.62) than in nonabstinent smokers or nonsmokers (rs = -.15 and .19, respectively). Thus, we propose that trait anxiety was not the primary determinant of between-groups differences observed in startle probe P3 suppression, but rather that it exacerbated the effects of tobacco abstinence on reactivity to unpleasant stimuli (cf. Baker et al., 2004).

In addition to its relationship to P3 suppression, trait anxiety was also related to individual differences in potentiated startle, in that abstinent smokers showed a significant negative correlation between trait anxiety scores and fear-potentiated startle. Since the abstinent group also showed the highest overall level of trait anxiety, this finding suggests that especially high trait anxiety was associated with decreased fear-potentiated startle. At first blush, this appears to be the reverse of the relationship one would expect between anxiety and the expression of fear to negative emotional cues. Interestingly, however, low levels of fear-potentiated startle have previously been reported in participants with high STAI-Trait scores and in patients diagnosed with

panic disorder with agoraphobia and generalized anxiety disorder (Lang & McTeague, 2009). A possible explanation for this relationship lies in the interplay between fear conditioning to explicit cues (i.e., the arousing pictures) versus contextual cues (i.e., the lab in which the experiment was conducted). That is, in contrast to the reduction in potentiated startle to pictorial cues (Lang & McTeague, 2009), startle reactivity to contextual cues is elevated in the anxiety diagnoses that are associated with high trait anxiety scores (i.e., panic disorder and post-traumatic stress disorder; Grillon et al., 2008; Grillon et al., 2009). Moreover, recent preclinical work indicates that these two phenomena may be causally related: high contextual fear, mediated by excessive activation of the bed nucleus of the stria terminalis in the basal forebrain, disrupts acquisition of fear to explicit cues (Duvarci, Bauer, & Pare, 2009; Radke, 2009). Although not measured directly in this study, potentiated startle to contextual cues is also elevated during smoking abstinence (Grillon et al., 2007). Thus, it is likely that the most anxious of the abstinent smokers in the current study developed the highest levels of fear to the context in which the experiment took place, and that exaggerated contextual fear in turn reduced expression of fear-potentiated startle.

Potential limitations and future directions

The main conclusions drawn from our study are that abstinent smokers are more reactive to unpleasant stimuli and that those who show increased craving are also more reactive to tobacco stimuli. These conclusions need to be interpreted, however, within the context of this study's limitation that the smokers we recruited were relatively young and light smokers compared to the participants in many other smoking studies.

This raises the concern that they may not have been truly "dependent" on nicotine (cf. Heatherton et al., 1991), making it difficult to generalize conclusions drawn from this sample to clinical populations of heavy smokers who are trying to quit. Arguing against this, several of our findings agree with those from studies of heavy smokers. Heavy smokers have been shown to rate tobacco pictures similarly to other high-arousal pleasant stimuli (Carter et al., 2006; Geier et al., 2000; Orain-Pelissolo et al., 2004) and to show reduced fear-potentiated startle during unpleasant pictures after acute nicotine administration (Cinciripini et al., 2006). Nonabstinent, heavy smokers also exhibit suppressed startle probe P3 magnitude during tobacco pictures (Versace et al., 2010), but the effects of abstinence on startle probe P3 suppression has yet to be examined in heavy smokers.

A further issue related to the use of light smokers is whether the abstinence effects can be attributed to a "true" nicotine withdrawal episode. Again, this concern is mitigated by the similarity of our findings to those in heavy smokers, and the results from several studies suggesting that signs and symptoms of tobacco withdrawal and difficulty quitting are evident in even the lightest (Davies, Willner, & Morgan, 2000; O'Loughlin et al., 2003; Sayette, Martin, Wertz, Shiffman, & Perrott, 2001) and most inexperienced (DiFranza et al., 2000; Kassel et al., 2007) smokers. Given the vulnerability of adolescent (Chassin, Presson, Rose, & Sherman, 1996) and college-age (Wetter et al., 2004) smokers to transition from light to heavy smoking, it is important to continue including participants from these groups in tobacco cue reactivity studies.

A final, potential concern is our adoption of a between-subjects abstinence manipulation, which prevents us from completely eliminating the possibility that abstinence effects reported here were due to differences in participant characteristics that were unrelated to abstinence. For example, abstinent smokers scored significantly higher than nonabstinent smokers on the impulsivity scale of the EASI, demonstrating that factors other than abstinence can differentiate between groups in a between-subjects design. However, no significant main effects or interactions involving median splits on EASI-I scores were found, and none of the correlations between EASI-I scores and physiological measures approached significance. The lack of significant differences between nonsmokers, nonabstinent smokers, and abstinent smokers on all other Visit 1 questionnaire scores is also encouraging, suggesting that smoking status was the largest and most important difference between groups. Furthermore, a between-subjects approach to smoking withdrawal studies is advantageous in that it avoids difficulties associated with between-sessions habituation to the pictures and the startle stimuli (Ornitz & Guthrie, 1989), participant fatigue and attrition, and the need to address order effects of the abstinent and nonabstinent condition.

Notwithstanding these potential concerns, the results of this study are supportive of several predictions of negative-affect reduction models of tobacco addiction. These models predict that smoking reduces negative affect (Baker et al., 2004; Koob & Le Moal, 2008; Solomon & Corbit, 1973; Watkins, Koob et al., 2000), which is supported by our finding of less fear-potentiated startle in nonabstinent smokers than abstinent smokers. A related prediction is that tobacco withdrawal increases emotional responses

to unpleasant stimuli (Baker et al., 2004), which is supported by our finding of increased startle probe P3 suppression during unpleasant pictures in abstinent smokers. Affect-management models also predict that tobacco abstinence selectively increases attention to unpleasant and tobacco cues, but not pleasant or neutral cues (D. G. Gilbert, 1997), which is also supported by our P3 suppression results. Thus, the findings of this study highlight the need for further developing smoking-cessation therapies that target the negative affective consequences of tobacco use and abstinence. Moreover, the methods used in this study – measuring startle amplitude and P3 suppression to the startle stimulus – appear to be highly sensitive to these consequences and may therefore prove extremely useful tools in the development of new treatment strategies.

CHAPTER 4: EMOTIONAL REACTIVITY TO EMOTIONAL AND SMOKING CUES DURING TOBACCO ABSTINENCE: LATE POSITIVE POTENTIALS AND PERIPHERAL PSYCHOPHYSIOLOGY

Negative reinforcement is thought to be a critical factor in the maintenance of cigarette smoking (Baker et al., 2004; Koob & Le Moal, 2008; Solomon & Corbit, 1973). Affective signs of tobacco withdrawal, such as increased anxiety and cigarette craving, emerge shortly after the last cigarette is smoked and peak within the first 2 days of abstinence (Hughes, 1992, 2007). Many smokers relapse during this period of heightened negative affect (Gritz et al., 1991), and there is a considerable body of evidence that relapse is precipitated by increased anxiety, stress, or craving (Allen et al., 2008; Piasecki et al., 1998; Piasecki et al., 2000; Shiffman et al., 1996) and that smoking a cigarette is anxiolytic (Parrott, 1993, 1995). Thus, interventions that reduce withdrawal-induced negative affect or the anxiolytic potency of smoking may be especially effective smoking cessation therapies (e.g., R. West et al., 2008).

The emotional consequences of tobacco use are thought to arise from the effects of nicotine on brain motivational systems (Watkins, Koob et al., 2000). Detailed understanding of the neurobiology of smoking-related emotional responses may therefore facilitate the development of therapies that reduce these responses. However, little is known about the neurobiology of smoking in humans, primarily because most studies of affect and smoking have used self-report as the only measure of emotional response. Psychophysiological measures are thought to be better indicators of the biological basis of motivation (Lang et al., 1990; Lang, Bradley, & Cuthbert, 1998), and

therefore to offer a promising strategy in the study of the neurobiology of tobacco addiction.

One paradigm for studying the psychophysiology of tobacco addiction is an extension of the emotional cue reactivity task developed by Lang and colleagues (e.g., Lang, Greenwald, Bradley, & Hamm, 1993; Schupp et al., 2004). In studies of smoking cue reactivity, psychophysiological responses are recorded while participants view pictures from four categories: pleasant, neutral, unpleasant, and tobacco-related. In comparison to nonsmokers, tobacco pictures appear to be pleasant and arousing for nonabstinent smokers as evidenced by self-report (Orain-Pelissolo et al., 2004), eyeblink startle inhibition (Cinciripini et al., 2006; Geier et al., 2000; but see Orain-Pelissolo et al., 2004), startle probe P3 suppression (Versace et al., 2010), and elevated late positive potentials (LPPs; Warren & McDonough, 1999), zygomatic electromyography (EMG) responses (Geier et al., 2000), and skin conductance responses (SCRs; Orain-Pelissolo et al., 2004). Abstinence periods of 12 h have little effect on smokers' physiological responses to tobacco pictures (Geier et al., 2000; McDonough & Warren, 2001). Responses to pleasant and neutral pictures do not differ between smokers and nonsmokers regardless of deprivation status (Cinciripini et al., 2006; Geier et al., 2000; D. G. Gilbert et al., 2004; D. G. Gilbert et al., 2007; Versace et al., 2010), but there is evidence that intranasal or transdermal nicotine decreases abstinent smokers' emotional reactivity to unpleasant pictures (Cinciripini et al., 2006; D. G. Gilbert et al., 2004; D. G. Gilbert et al., 2007; Rzetelny et al., 2008).

Two factors should be noted regarding the abstinence manipulations in previous cue reactivity studies. First, the deprivation period lasted only 12 h, which is before the peak in withdrawal symptom severity. Second, cue reactivity was tested at the end of the deprivation period and smoking was permitted immediately after the test session. In the current study, cue reactivity was measured 24 h into a 48 h abstinence period. This abstinence manipulation may be more clinically relevant, because smokers who are trying to quit must remain abstinent for extended periods of time in spite of being exposed to tobacco cues. In fact, contemporary negative reinforcement models of tobacco abstinence predict that tobacco cues may be aversive in these circumstances (Baker et al., 2004), a phenomenon similar to that of frustrative non-reward, observed in animals denied reinforcement in the presence of cues where reinforcement was previously delivered (Drobes et al., 2001; Hull, 1943). It is therefore possible that psychophysiological responses to smoking cues presented in the middle of an abstinence period will resemble responses to unpleasant pictures, rather than pleasant pictures.

In this manuscript, we report LPPs, facial EMG, and SCRs to cue onset in nonsmokers, nonabstinent smokers, and abstinent smokers who were 24 h into a 48 h deprivation period. A previous manuscript reported eyeblink startle amplitude and P3 suppression to startle probes delivered during the cues (Chapter 3).

Method

Participants

Participants were recruited via advertisements posted throughout the university campus and the surrounding community and via campus-based websites. These advertisements solicited the participation of nonsmokers and daily smokers between the ages of 18 and 35 who were not currently seeking treatment for smoking cessation. A total of 81 participants meeting these criteria gave written, informed consent to participate in the study. The current analyses are limited to daily smokers with baseline expired-air carbon monoxide (CO) levels of at least 7 ppm and nonsmokers who reported never smoking a cigarette and who had baseline CO levels of less than 7 ppm. The CO criteria used in this study have been shown to reliably differentiate between nonsmokers and smokers and to reliably detect tobacco abstinence (e.g., Marrone et al., 2010). Other exclusion criteria included current diagnosis of major psychiatric illness, medical conditions or use of medications that would influence psychophysiological recording, and current use of over the counter or prescription stop-smoking remedies or current participation in stop-smoking counseling. This resulted in sample sizes of 19 nonsmokers (11 female), 17 smokers assigned to the nonabstinent condition (8 female), and 18 smokers assigned to the abstinent condition (9 female). Participants had a choice of $50 or extra credit for their psychology course as compensation for completing all three visits, and smokers assigned to the abstinent group who met criteria for abstinence verification (see below) received an additional cash bonus of $25 or additional course credit.

All psychophysiological data from one female nonsmoker were lost due to equipment failure, two smokers (1 female) assigned to the abstinent condition dropped out of the study prior to the psychophysiological recording session, and one male smoker assigned to the abstinent condition failed to maintain abstinence for 24 h prior to the psychophysiological recording session, leaving final sample sizes of 18 nonsmokers (10 female), 17 nonabstinent smokers (8 female), and 15 abstinent smokers (8 female). Data from 5 abstinent smokers (4 female) who were successfully abstinent at the start of Visit 2 but not at the start of Visit 3 were included in this analysis, as the primary interests of this study were the psychophysiological responses collected during Visit 2. The psychophysiological responses of this subgroup did not significantly differ from those of smokers who remained abstinent for the entire 48 hour period.

Stimuli

Sixty full-color, 1024 x 768 pixel stimuli were presented via a 41 x 31 cm LCD video monitor (Model 2001FP, Dell, Round Rock, TX), subtending a horizontal viewing angle of 26 degrees. These stimuli were classified according to four categories, with 15 pictures in each category: tobacco, pleasant, neutral, and unpleasant. The pleasant, neutral, and unpleasant stimuli were selected from the International Affective Picture System (Lang et al., 1999) and the tobacco stimuli were selected from a set of pictures collected by the authors from internet searches and print media and from a set provided by the lab of Paul Cinciripini (Carter et al., 2006). All stimuli used in this

study[3] were previously normed on measures of affective valence, arousal, and dominance in our laboratory. Detailed analyses of the ratings from the current sample are reported in Chapter 3. Briefly, pleasant and unpleasant pictures were both rated as more arousing than neutral pictures in all three groups of participants, and smokers rated tobacco pictures as more pleasant and arousing than did nonsmokers.

Psychophysiological measures

The electroencephalogram was recorded using 4-mm diameter Ag-AgCl electrodes at the Fz, Cz, and Pz electrode sites of the International 10-20 System (Jasper, 1958), referenced to linked mastoids (models E271-LS for scalp leads and E220N-LS for mastoid leads, In Vivo Metric, Healdsburg, CA). EEG traces were amplified and bandpass-filtered (0.1 - 40 Hz) using a Coulbourn (Whitehall, PA) V75-08 EEG amplifier. For purposes of correcting eye movement artifacts in the EEG data, vertical electrooculogram (vEOG) was obtained by splitting the signal from the orbicularis EMG electrode placed directly below the pupil of the subject's non-dominant eye and the corrugator EMG electrode placed directly above the pupil. The vEOG signal obtained was amplified and bandpass-filtered (0.1 - 40 Hz) using a Coulbourn V75-04 bioamplifier. An additional electrode placed on the forehead provided the signal ground for the EEG, EMG, and vEOG channels. EEG and vEOG were digitized at a sampling rate of 125 Hz using an analog to digital converter

[3] The IAPS pictures used were: Pleasant: 4606, 4611, 4651, 4653, 4687, 4694, 7330, 7410, 7450, 7470, 7488, 8034, 8117, 8499, 8502; Neutral: 2383, 2516, 2880, 2980, 5531, 5740, 7050, 7080, 7095, 7170, 7175, 7179, 7205, 7550, 7705; Unpleasant: 1051, 1270, 1300, 1930, 2095, 6022, 6200, 6243, 6244, 6260, 8480, 9042, 9290, 8342, 9592. The tobacco stimuli from Carter et al. (2006) were: 402, 403, and 414. The remaining smoking stimuli were from Engelmann and Cuthbert (2008): S254, S359, S369, S377, S413, S435, S441, S454, S457, S460, S466, and S479. These stimuli are available upon request from the author.

(LabMaster DPCI, Scientific Solutions, Mentor, OH) and a computer running VPM software (Cook, 2003; Cook et al., 1987)

Corrugator supercilii, zygomaticus major, and orbicularis oculi EMG were recorded using 4-mm diameter Ag-AgCl electrodes (E220N-LS, In Vivo Metric) using the placements recommended by Fridlund and Cacioppo (1986). Facial EMG signals were amplified and bandpass filtered (90 - 1000 Hz) using Coulbourn V75-04 bioamplifiers, smoothed using a Coulbourn V76-24 contour-following integrator with a time constant setting of 200 ms for corrugator and zygomatic EMG and 50 ms for orbicularis EMG, and digitized at sampling rate of 20 Hz.[4]

Skin conductance level was recorded from the hypothenear eminence of the palm of the non-dominant hand using two 8-mm diameter Ag-AgCl electrodes (E224A-LS, In Vivo Metric) filled with 0.5% NaCl electrode paste (TD-246, Discount Disposables, St. Albans, VT). The signal was amplified by a Coulbourn V71-23 isolated skin conductance coupler using DC coupling and an input range of 0 - 40 μS and digitized at a sampling rate of 20 Hz.

Procedure

Participants came to the laboratory for three sessions. During Visit 1, all participants completed several questionnaire measures to assess personality traits, anxiety, withdrawal symptoms, and cigarette craving, and provided breath samples for baseline CO measurement. CO level was defined as the mean of two samples taken using a PiCO Smokerlyzer (Bedfont Scientific USA, Williamsburg, VA). Smokers

[4] The time constant for orbicularis EMG was shorter than those for corrugator and zygomatic EMG because the orbicularis electrodes were also used to measure the eyeblink startle reflex.

were then randomly assigned to the nonabstinent or abstinent condition, and were instructed, respectively, to smoke as normal or to abstain from cigarettes and all other products containing nicotine or tobacco beginning 24 h prior to the start of Visit 2 through the end of Visit 3. Visits 2 and 3 were scheduled 24 h apart, within 3 weeks of Visit 1. Visits 1, 2 and 3 lasted approximately 1 h, 1.5 h, and 1 h, respectively.

Upon arrival at the laboratory for Visit 2, smokers in the nonabstinent group were asked to smoke one of their regular cigarettes and smoking status was verified using expired-air CO. Specifically, smokers assigned to the abstinent condition who reported no cigarette consumption 24 h prior to the start of the visit and had CO levels less than 7 ppm or half of their baseline value (whichever was lower) were classified as successfully abstinent. All participants then completed a small set of questionnaires to assess state anxiety, withdrawal symptoms, and cigarette craving. Following questionnaire administration, the psychophysiological recording electrodes were attached, and a 3-min baseline measurement was obtained while participants relaxed with their eyes closed. Then, the picture-viewing task began. This task consisted of a short practice block (4 picture trials, 1 from each category) followed by two full picture-viewing blocks, each consisting of 30 picture trials, presented in a random order with respect to picture category. A total of four picture orders were used, distributed randomly across subjects to reduce the likelihood that order effects would influence the results.

At the start of each block, the participant was instructed to pay attention to the pictures, told that the sensors were going to measure their "physiological reactions to

the pictures," and that they could ignore the loud noises that were presented during several of the pictures. On each trial, a picture was presented for 6 s, followed by an intertrial interval (ITI) ranging from 18-24 s during which a gray screen was presented. Acoustic startle probes (50-ms, 95-dB white noise bursts) were presented 2.5 or 4.5 s after picture onset during 12 of the 15 pictures from each content category and 8, 10, or 12 s after onset of 12 ITIs (startle data are reported in Chapter 3). At the conclusion of the picture-viewing task, the electrodes were removed while the participant completed a brief questionnaire, followed by dismissal until Visit 3.

The beginning of Visit 3 used the same procedure as the beginning of Visit 2 (i.e., nonabstinent smokers smoke, CO measurement, and questionnaires). All participants then completed two picture memory tasks (recall and recognition) and a picture rating task, during which they provided valence, arousal, and dominance ratings of each picture using the self-assessment manikin (Hodes et al., 1985). The results of this task are reported in Chapter 3. At the conclusion of the picture rating task, participants were debriefed, compensated, and dismissed.

Physiological data reduction

For each subject, raw EEG waveforms were digitally filtered using infinite impulse response filters with a high pass of 0.1 Hz and a low pass of 40 Hz, followed by extraction of epochs from 3 s before picture onset through 3 s after picture offset. Epochs with off-scale or flat EEG or vEOG traces or with large (> 300 μV) deflections in the raw EEG were rejected, followed by eye movement correction using the algorithm of Gratton, Coles, and Donchin (1983) implemented in Fortran 77 (Miller et

al., 1988). The epochs from individual trials were then averaged according to picture category and baseline corrected by subtracting the mean of the 120-ms window immediately preceding picture onset from all points in the waveform (Cuthbert, Schupp, Bradley, Birbaumer, & Lang, 2000; Schupp et al., 2004). The LPP was quantified as the average value of the baseline-corrected waveform in two windows, lasting from 400-700 and 700-1000 ms after picture onset (Schupp et al., 2004). Equipment failure resulted in the loss of LPP data for 2 male nonsmokers, resulting in final sample sizes for LPP data of 16 nonsmokers, 17 nonabstinent smokers, and 15 abstinent smokers.

Facial EMG traces were extracted in 0.5-s epochs from 1 s before picture onset through picture offset. Epochs during which a startle probe was delivered were excluded from further analysis to avoid contamination of the EMG response to the picture with the startle response. Trials with raw EMG values greater than 40 μV were discarded, as were trials with excessive noise, defined as trials with more than 3 epochs differing from that trial's mean value by more than 3 standard deviations. The EMG response to each picture was quantified as the mean of the epochs during picture presentation minus the mean of the 1-s baseline, and these scores were averaged according to picture category. In cases where more than 30% of trials were rejected due to artifact, the subject was excluded from further analysis. This resulted in the loss of orbicularis data from 1 male nonsmoker and 1 female abstinent smoker, corrugator data from 2 nonsmokers (1 female) and 1 female nonabstinent smoker, and zygomatic data from 2 male nonabstinent smokers and 1 female abstinent smoker. Thus, total sample sizes for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively, were

17, 17, and 15 for orbicularis, 16, 16 and 15 for corrugator, and 18, 15, and 14 for zygomatic.

Raw skin conductance levels in μS were extracted in 0.5-s epochs from 0.5 s before picture onset through picture offset. All epochs were baseline corrected via subtraction of the value of the pre-onset epoch. The baseline-corrected waveforms were then transformed by adding 1 to each data point and taking the base-10 logarithm of that value. The purpose of this transformation was to improve the normality of the skin conductance distribution and to eliminate trials on which no skin conductance response occurred (i.e., negative change from baseline; Lang et al., 1993). The SCR to each picture was then defined as the peak value of the transformed data within 3 s of picture onset (i.e., before SCRs to the startle probe emerged). The SCRs were range corrected such that the maximum response for each subject was defined as 100% SCR, and other responses were measured as a percentage of that maximum (Lykken, 1972). The range corrected SCR scores were then averaged according to picture category and subjected to statistical analysis.

Statistical analysis

The focus of these analyses was the difference in emotional response between nonsmokers, nonabstinent smokers, and abstinent smokers. Thus, after confirming that there were no significant differences between groups in responses to neutral pictures using one-way ANOVA, psychophysiological responses were analyzed by computing difference scores for each subject's mean response to each category of emotional picture (tobacco, pleasant, and unpleasant) from their mean response to neutral pictures.

[Emotional – neutral] difference scores were entered into group x picture category ANOVAs, where significant main effects of group would be indicative of differences in overall emotional reactivity between groups and significant group x category interactions would be indicative of between-group differences in response to at least one specific picture category. Significant main effects of group were followed by Tukey HSD *post hoc* tests and significant interactions were followed by tests for simple effects of group within each picture category using between-subjects ANOVA followed by Tukey HSD (Maxwell & Delaney, 1990).

For corrugator and zygomatic EMG, the main effect of picture category was used to test whether the typically observed pattern of EMG response was observed in this sample. For these responses, we expected a significant main effect, with the effects being due to greater corrugator responses to unpleasant pictures than to pleasant pictures and greater zygomatic responses to pleasant pictures than to unpleasant pictures (Lang et al., 1993). Orbicularis EMG, SCRs, and LPPs are typically elevated during both pleasant and unpleasant pictures, compared to neutral pictures (e.g., Bradley, Cuthbert, & Lang, 1990; Schupp et al., 2004). Thus, we did not expect a significant main effect of picture category for [emotional – neutral] difference scores for these measures. To verify that these responses were greater during pleasant and unpleasant pictures than during neutral pictures, we used one-sample *t*-tests to assess whether the mean difference scores were significantly greater than zero.

We were also interested in how individual differences in self-reported cigarette craving, withdrawal symptoms, and anxiety influenced psychophysiological responses

to emotional pictures. To this end, we assigned nonabstinent and abstinent smokers to subgroups based on median splits of [Visit 2 – Visit1] difference scores on the following measures: Factor 1 of the Questionnaire of Smoking Urges (QSU1; Tiffany & Drobes, 1991), a measure of cigarette craving, Factor 2 of the QSU (QSU2), a measure of desire to smoke to avoid withdrawal symptoms and negative affect (QSU2), the Minnesota Nicotine Withdrawal Scale (MNWS; Hughes & Hatsukami, 1998), and the State scale of the State-Trait Anxiety Inventory (STAI-State; Spielberger, 1979). We also assigned participants to subgroups based on their Visit 1 scores on the Trait scale of the STAI. For each questionnaire measure, subgroup assignment was entered into the ANOVAs described above as an additional between-subjects factor.

Statistical analyses were conducted using SYSTAT 12 Software (Chicago, IL). The significance criterion for all ANOVAs was set to $p < .05$. Multivariate test statistics (Wilks λ and its approximate F statistic) were used to assess the significance of all effects involving the within-subjects factor of picture category (Johnson & Wichern, 2002).

Results

Participant Characteristics and Subjective Ratings of Abstinence

Participants were young (mean age: 21.5 years, *SEM*: 0.5) and the smokers were relatively light smokers, smoking an average of 10.9 ± 1.1 cigarettes per day for 6.3 ± 0.8 years, with an average score on the Fagerström Test for Nicotine Dependence (Heatherton et al., 1991) of 3.1 ± 0.3 out of a maximum score of 10. Baseline expired-air CO levels were 2.4 ± 0.3 ppm for nonsmokers, 12.6 ± 1.5 for smokers assigned to

the nonabstinent group, and 13.5 ± 1.2 for smokers assigned to the abstinent group. CO levels remained low in nonsmokers and high in nonabstinent smokers at the start of Visits 2 and 3 (Visit 2: Nonsmokers: 2.0 ± 0.2, Nonabstinent Smokers: 13.6 ± 1.8; Visit 3: Nonsmokers: 1.8 ± 0.1, Nonabstinent Smokers 13.6 ± 2.2 ppm). Abstinent smokers' CO levels fell during Visit 2 (2.4 ± 0.3 ppm) and remained low during Visit 3 (4.5 ± 1.1 ppm). Scores on the MNWS, both factors of the QSU, and STAI-State increased from Visit 1 to Visit 2 and remained high through Visit 3 in abstinent smokers, whereas these scores did not significantly change across sessions in nonabstinent smokers or nonsmokers. Additional details on participants' smoking histories, CO analysis, and questionnaire scores are presented in Chapter 3.

Late Positive Potential: 400-700 ms after picture onset

Grand average waveforms are presented in Figure 4-1. The topography of the waveforms observed in the current study closely resembled those observed in other studies of ERPs to emotional pictures (Cuthbert et al., 2000; Schupp et al., 2004). Window averages for the interval lasting from 400-700 ms after picture onset are presented in Figure 4-2. One-way ANOVA for group found no significant differences between responses to neutral pictures at any electrode site [all Fs(2,45) < 1.4, all ps > .1]. One-sample t-tests for [pleasant – neutral] and [unpleasant – neutral] difference scores found that these scores were significantly greater than zero at all electrode sites [all ts(47) > 2.04, all ps < .05]. This indicates that LPP magnitude was greater during pleasant and unpleasant pictures than during neutral pictures. LPPs to tobacco pictures were also significantly greater than those to neutral pictures at Pz, as indicated by

difference scores significantly greater than zero [$t(47) = 3.34$, $p < .01$]. This difference approached significance at Cz [$t(47) = 1.95$, $p = .06$], but not at Fz [$t(47) = 1.64$, $p > .1$].

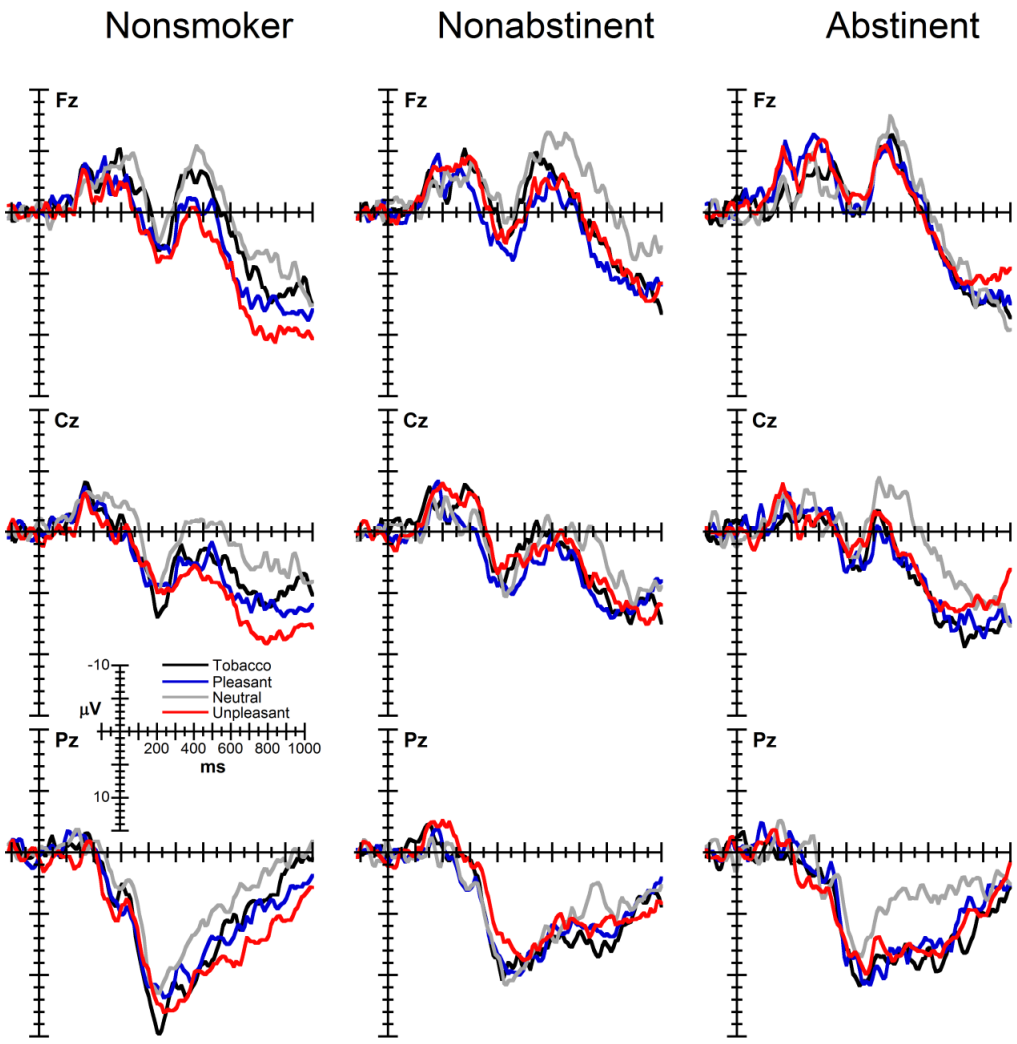

Figure 4-1. Grand average waveforms for event-related potentials to tobacco, pleasant, neutral, and unpleasant pictures. The data plotted range from 120 ms before through 1000 ms after picture onset, where 0 ms represents picture onset. The EEG was measured in μV, where negative values are plotted above the *x*-axis and positive values are plotted below the *x*-axis. Electrode sites (Fz, Cz, and Pz) are plotted in *rows* and groups (nonsmokers, nonabstinent smokers, and abstinent smokers) are plotted in *columns*. *n*s = 16, 17, and 15 for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively.

[Emotional – neutral] difference scores were analyzed using group x picture category ANOVAs. At the Pz electrode site, there was a significant main effect of group [$F(2,45) = 3.32$, $p < .05$], but no significant main effect of picture category

[$F(2,44) < 1$, $p > .1$, $\lambda = .98$] or group x picture category interaction [$F(4,88) < 1$, $p > .1$, $\lambda = .97$]. Tukey *post hoc* tests indicated that nonabstinent smokers had lower [emotional – neutral] difference scores than abstinent smokers, and that nonsmokers did not differ significantly from either group of smokers. At Fz and Cz, group and picture category main effects and group x picture category interactions were not statistically significant (all Fs < 1.7, all ps > .1).

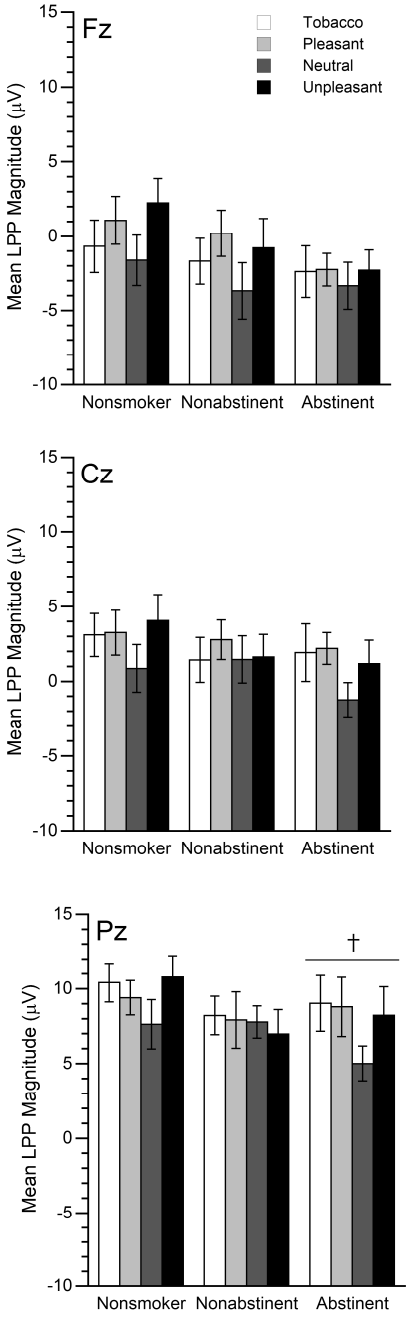

Figure 4-2. Mean LPP magnitude from 400-700 ms after onset of tobacco, pleasant, neutral, and unpleasant pictures at Fz (*upper panel*), Cz (*middle panel*), and Pz (*lower panel*) electrode sites in nonsmokers, nonabstinent smokers, and abstinent smokers. *Error bars* depict the mean ± 1 *SEM*. *n*s = 16, 17, and 15 for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively. † indicates that the mean [emotional – neutral] difference score, averaged across tobacco, pleasant, and unpleasant pictures, was significantly different from the same score in the nonabstinent group ($p < .05$, Tukey HSD *post hoc* test).

Late Positive Potential: 700-1000 ms after picture onset

Window averages for the interval lasting from 700 to 1000 ms after picture onset are presented in Figure 4-3. One-way ANOVA for group found no significant differences in responses to neutral pictures [all Fs(2,45) < 2.4, all ps > .1], and one sample t-tests found that the [tobacco – neutral], [pleasant – neutral], and [unpleasant – neutral] difference scores were significantly greater than zero at all three electrode sites [all ts(47) > 2.0, all ps < .05]. Group x picture category ANOVAs on these [emotional – neutral] difference scores found no significant main effects of group [all Fs(2,45) < 2.0, all ps > .1], picture category [all Fs(2,44) < 1, all ps > .1, all λs > .97], or group x picture category interaction [all Fs(4,88) < 1.5, all ps > .1, all λs > .88] at any electrode site.

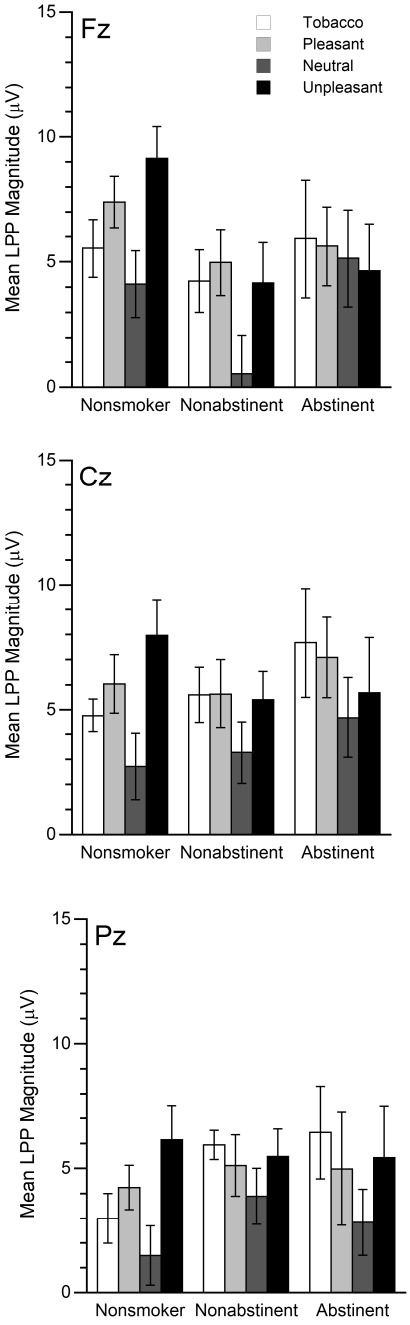

Figure 4-3. Mean LPP magnitude from 700-1000 ms after onset of tobacco, pleasant, neutral, and unpleasant pictures at Fz (*upper panel*), Cz (*middle panel*), and Pz (*lower panel*) electrode sites in nonsmokers, nonabstinent smokers, and abstinent smokers. *Error bars* depict the mean ± 1 *SEM*. *n*s = 16, 17, and 15 for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively.

Facial EMG and Skin Conductance

Mean corrugator, zygomatic, orbicularis, and skin conductance responses are presented in Figure 4-4. One-way ANOVAs found no between-groups differences in responses to neutral pictures for any measure [all Fs < 1.9, all ps > .1].

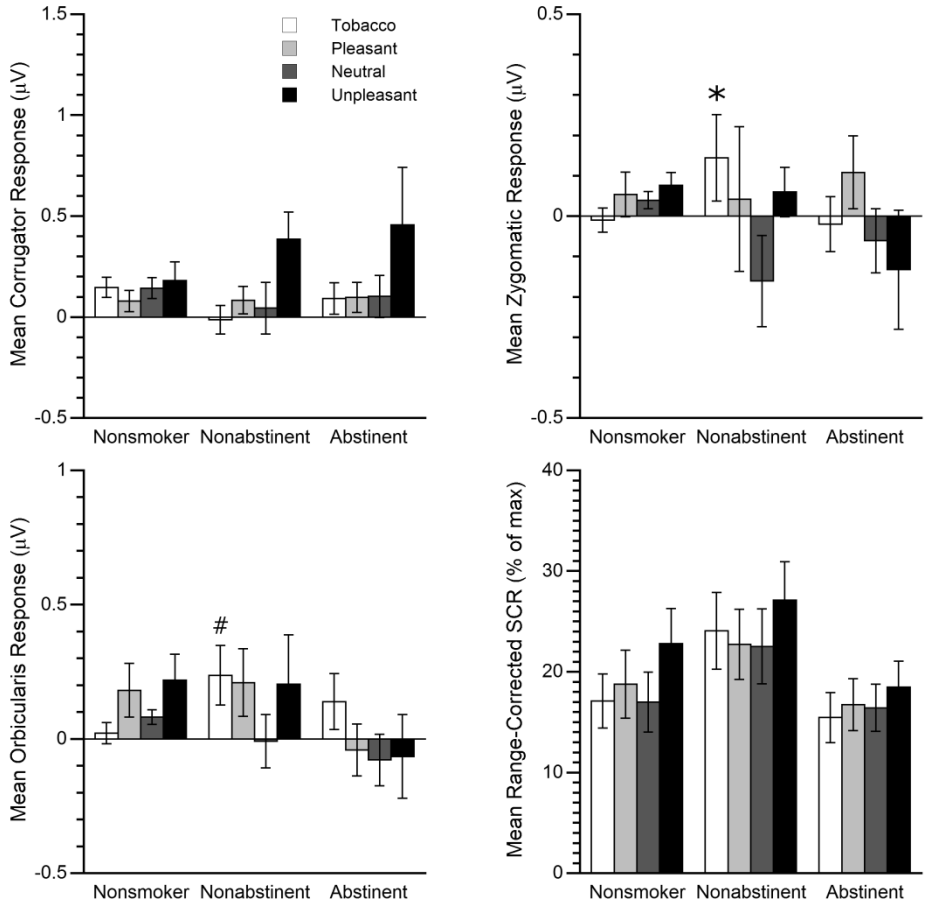

Figure 4-4. Facial EMG and SCRs to tobacco, pleasant, neutral, and unpleasant pictures in nonsmokers, nonabstinent smokers, and abstinent smokers. *Upper left:* Corrugator EMG responses, *n*s = 16, 16, and 15 for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively. *Upper right:* Zygomatic EMG responses, *n*s = 18, 15, and 14. *Lower left:* Orbicularis EMG responses, *n*s = 17, 17, and 15. *Lower right:* Range-corrected SCRs, *n*s = 18, 17, and 15. In all panels, *error bars* depict the mean ± 1 SEM. # ($p < .1$) and * ($p < .05$) indicate that the mean [tobacco – neutral] difference score in the marked group was significantly or marginally significantly different from the same score in the nonsmoker group, measured using Tukey HSD *post hoc* tests.

For corrugator EMG, the main effect of picture category was statistically significant [$F(2,43) = 4.19$, $p < .05$, $\lambda = .84$], but the main effect of group [$F(2,44) < 1$,

$p > .1$] and the group x picture category interaction [$F(4,86) = 1.18$, $p > .1$, $\lambda = .90$] were not significant. Simple effects tests indicated that, as expected, the main effect of picture category was due to greater corrugator responses to unpleasant pictures than to tobacco pictures and pleasant pictures [Fs$(1,46) > 7.73$, ps $< .01$]. For zygomatic EMG, the main effects of group [$F(2,44) = 1.94$, $p > .1$] and picture category [$F(2,43) < 1$, $p > .1$, $\lambda = .98$] and their interaction [$F(4,86) = 1.17$, $p > .1$, $\lambda = .90$] were not statistically significant. Although zygomatic EMG responses to tobacco pictures were greater in nonabstinent smokers than in nonsmokers [one way ANOVA for group on (tobacco – neutral) difference scores: $F(2,44) = 3.64$, $p < .05$], this effect must be interpreted cautiously because of the lack of a significant interaction.

Unexpectedly, [pleasant – neutral] and [unpleasant – neutral] difference scores for orbicularis EMG were not significantly different than zero [pleasant: $t(47) = 1.70$, $p = .1$; unpleasant: $t(47) = 1.41$, $p > .1$]. However, the [tobacco – neutral] difference score was significantly different than zero [$t(47) = 2.23$, $p < .05$], which was indicative of greater overall orbicularis responses to tobacco pictures than to neutral pictures. The group x picture category ANOVA did not find significant main effects of group [$F(2,45) < 1$, $p > .1$], picture category [$F(2,44) < 1$, $p > .1$, $\lambda = .99$], or their interaction [$F(4,88) = 1.08$, $p > .1$, $\lambda = .91$]. Similar to the result for zygomatic EMG, there was a significant main effect of group on [tobacco – neutral] difference scores [$F(2,45) = 3.23$, $p < .05$], despite the lack of a significant group x picture category interaction. This effect was the result of larger orbicularis EMG responses to tobacco pictures in

nonabstinent smokers than in nonsmokers, as indicated by a marginally significant Tukey *post hoc* test ($p = .06$).

For skin conductance, [unpleasant – neutral] difference scores were significantly greater than zero [$t(49) = 4.01$, $p < .001$], but [pleasant – neutral] and [tobacco – neutral] difference scores were not [ts(49) < 1.1, ps > .1]. These differences between picture categories were reflected in a significant main effect of picture category in the group x category ANOVA [$F(2,46) = 7.79$, $p < .01$, $\lambda = .75$]. The main effect of group [$F(2,47) < 1$, $p > .1$] and the group x picture category interaction [$F(4,92) < 1$, $p > .1$, $\lambda = .93$] were not statistically significant.

QSU Scores and Psychophysiology

When median-split subgroup assignments for self-report measures were included in the ANOVAs, significant effects involving both factors of the QSU emerged. The QSU1 subgroup x category interaction was statistically significant for the 400-700 ms LPP at Cz [$F(2,27) = 4.91$, $p < .05$, $\lambda = .73$] and Pz [$F(2,27) = 3.52$, $p < .05$, $\lambda = .79$], but only approached significance at Fz [$F(2,27) = 2.92$, $p = .07$, $\lambda = .82$]. At Cz and Pz, this interaction was the result of a simple effect of QSU1 subgroup assignment on responses to pleasant pictures [Fs(1,30) > 3.4, ps < .05], but not tobacco or unpleasant pictures [Fs(1,30) < 1, ps > .1]. Specifically, participants who scored above the median QSU1 score within their abstinence condition had the highest [pleasant – neutral] difference scores for the LPP (Figure 4-5, left panel). The abstinence group x QSU2 subgroup interaction was statistically significant for the 400-700 ms LPP at Cz [$F(1,28) = 4.26$, $p < .05$], but not at Fz [$F(1,28) = 2.27$, $p > .1$] or Pz

[$F(1,28) < 1$, $p > .1$]. This interaction was due to a simple effect of abstinence group in participants who scored above the median QSU2 score [$F(1,12) = 9.02$, $p < .05$], but not in those who scored below the median [$F(1,16) < 1$]: For those with scores above the median, the average LPP difference score across tobacco, pleasant, and unpleasant pictures was greater in abstinent smokers than in nonabstinent smokers (Figure 4-5, right panel).

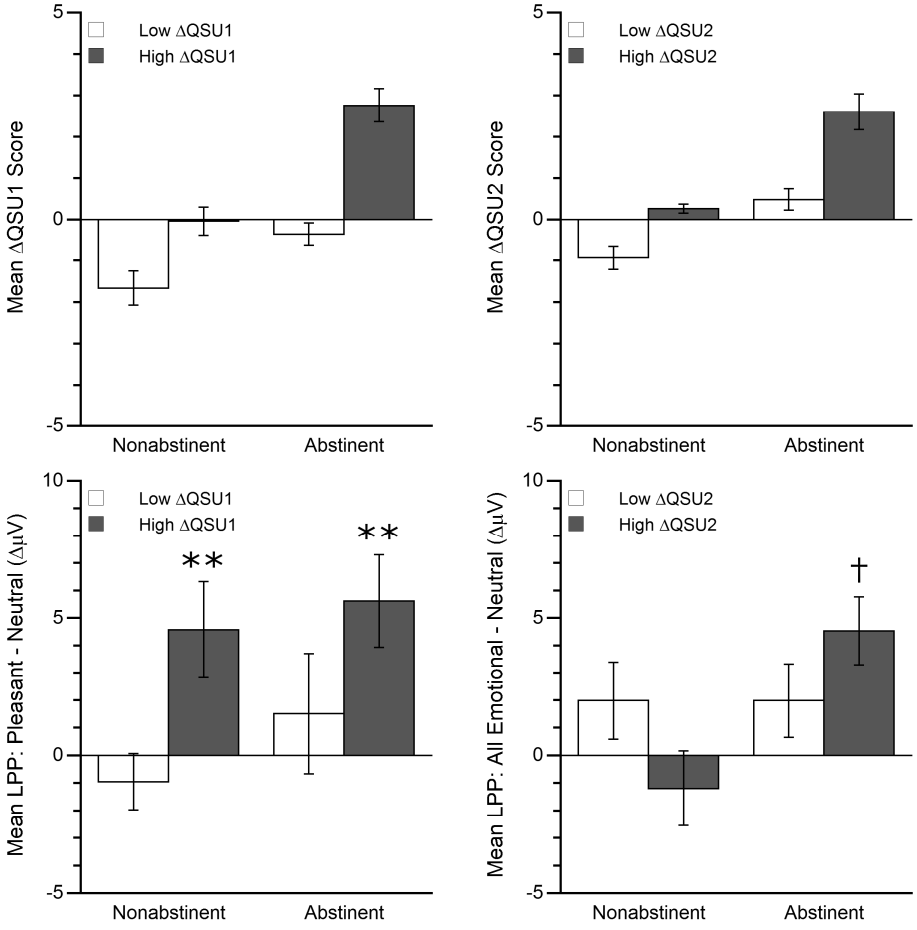

Figure 4-5. LPP magnitude from 400-700 ms after picture onset at the Cz electrode site in nonabstinent and abstinent smokers as a function of subgroup assignments based on median splits of QSU scores. *Upper left:* Change in QSU Factor 1 scores (cigarette craving) from Visit 1 to Visit 2 (ΔQSU1) as a function of abstinence and high vs. low ΔQSU1 subgroup assignment. *Upper right:* Change in QSU Factor 2 scores (desire to avoid withdrawal) from Visit 1 to Visit 2 (ΔQSU2) as a function abstinence and high vs. low ΔQSU2 subgroup assignment. *Lower left*: Mean [pleasant – neutral] difference in LPP magnitude as a function of abstinence and high vs. low ΔQSU1 subgroup assignment. *Asterisks* depict the simple effect of ΔQSU1 subgroup assignment, where the mean of the groups marked with ** differ significantly from the mean of the groups not marked with **, $p < .01$. *Lower right:* Mean overall [emotional (tobacco, pleasant, and unpleasant) – neutral] difference scores for LPP magnitude as a function of abstinence and high vs. low ΔQSU2 subgroup assignment. † Indicates a significant difference from the nonabstinent group within the same ΔQSU2 subgroup assignment, $p < .05$. In all panels, error bars depict the mean ± 1 *SEM*. Sample sizes for nonabstinent-low, nonabstinent-high, abstinent-low, and abstinent-high, respectively, were 10, 7, 8, and 7 for ΔQSU1 analysis (*left column*) and 9, 8, 9, and 6 for ΔQSU2 analysis (*right column*).

Discussion

The main finding from this study was that smokers who had a cigarette immediately prior to the psychophysiological recording session did not show the typical pattern of affect-modulation of the LPP in a window lasting from 400-700 ms after picture onset. Instead, their responses to arousing tobacco, pleasant, and unpleasant pictures did not differ from their responses to neutral pictures. This is in contrast to both nonsmokers and abstinent smokers, who showed elevated LPP magnitude to tobacco, pleasant, and unpleasant pictures.

The LPP is thought to reflect recruitment of cortical arousal systems in the occipital and posterior parietal cortices in response to motivationally relevant stimuli and is often interpreted as an index of attention to these stimuli (Cuthbert et al., 2000; Keil et al., 2002; Palomba, Angrilli, & Mini, 1997; Sabatinelli, Lang, Keil, & Bradley, 2007; Schupp et al., 2000; Schupp et al., 2004). Thus, it can be inferred that this attentional process and the patterns of neural activity that underlie it were drastically reduced in the earliest stages of picture processing in nonabstinent smokers. Because significant positivity was observed in the same time window in both nonsmokers and abstinent smokers, this effect appears to be due to the acute effects of smoking a cigarette rather than the effects of tobacco abstinence. As outlined below, this pattern of results is consistent with several predictions of affect-reduction models of tobacco addiction.

Modern negative-reinforcement (Baker et al., 2004) and affective processing (D. G. Gilbert et al., 2007) models of tobacco use propose that smoking reduces emotional

responses to aversive stimuli. Our finding that LPP magnitude to unpleasant pictures was reduced in nonabstinent smokers is consistent with this prediction. In the same sample of subjects used in the current analysis, fear-potentiated startle during unpleasant pictures was also reduced in nonabstinent smokers compared to both abstinent smokers and nonsmokers (Chapter 3). Startle probe P3 suppression, another measure of attention allocation to the pictures (Cuthbert et al., 1998; Schupp et al., 1997; Schupp et al., 2004), was also smaller in nonabstinent smokers than in abstinent smokers. There is also evidence from other laboratories using similar picture-viewing tasks that intranasal or transdermal nicotine administration in 12-h deprived smokers reduces fear-potentiated startle (Cinciripini et al., 2006) and the processing negativity (PN) ERP to unpleasant pictures (PN is similar to the LPP in that it is thought to reflect attention to the pictures; D. G. Gilbert et al., 2004). Collectively, these results support the prediction of negative reinforcement models of tobacco addiction that smoking decreases emotional reactivity to unpleasant pictures.

Affective models of tobacco addiction also predict that tobacco cues are motivationally relevant for smokers, but not for nonsmokers, and that abstinence increases the motivational significance of tobacco cues (e.g., Baker et al., 2004). One of these models also predicts that nicotine administration should reduce attention to tobacco cues (D. G. Gilbert et al., 2007). Our finding of significant late positivity to tobacco cues in abstinent but not in nonabstinent smokers supports this prediction, and other studies have found similar results (McDonough & Warren, 2001). Interestingly, our sample of nonsmokers had elevated LPPs during tobacco pictures, which is

inconsistent with previous studies that found no difference in LPP magnitude between tobacco and neutral pictures in nonsmokers (Littel & Franken, 2007; Warren & McDonough, 1999). One possible explanation is that, knowing that the study was about smoking, the nonsmokers treated the smoking pictures as "target stimuli," which resulted in elevated LPP magnitude (cf. Cacioppo, Crites, Berntson, & Coles, 1993).

Some affective models of tobacco addiction predict that one of the acute effects of smoking is to bias attention toward pleasant stimuli (D. G. Gilbert et al., 2007; T. E. Robinson & Berridge, 1993; Sherman, Morse, & Baker, 1986); this would presumably be reflected in increased LPPs to pleasant pictures immediately after smoking. The reduction of late positivity to pleasant pictures in the current sample of nonabstinent smokers does not support this prediction. Other ERP studies have also failed to find evidence for increased attentional bias to pleasant pictures in nonabstinent smokers: Transdermal nicotine administration had no effect on PN to pleasant pictures (D. G. Gilbert et al., 2004) or P3 suppression to target stimuli presented during pleasant pictures (D. G. Gilbert et al., 2007).

Craving, withdrawal-avoidance, and the LPP

Interestingly, LPPs to pleasant pictures – but no other category – varied as a function of self-reported cigarette craving. The three subgroups (Nonabstinent-High ΔQSU1, Abstinent-Low ΔQSU1, and Abstinent-High ΔQSU1) with increased or unchanged craving scores from Visit 1 to Visit 2 all had positive [pleasant – neutral] LPP scores. The only subgroup to show decreased craving (Nonabstinent-Low ΔQSU1) was also the group that had negative [pleasant – neutral] LPP scores. This

suggests that decreased attention to pleasant pictures may reflect smoking-induced decreases in craving. This finding is somewhat consistent with the idea that drug craving produces attentional bias to appetitive stimuli (T. E. Robinson & Berridge, 1993) in that decreased craving was associated with a loss of such attentional bias. However, this speculative conclusion must be interpreted cautiously for several reasons, including the small sample sizes within each subgroup and the numerically (but not significantly) lower late positivity in the Abstinent-Low ΔQSU1 subgroup compared to the Nonabstinent-High ΔQSU1 subgroup, which had similar ΔQSU1 scores. Thus, further research into the relationship between craving and LPPs to pleasant pictures is needed.

Withdrawal-avoidance motivation (QSU2) change scores also interacted with LPPs. In this case, the interactions were for all motivationally relevant pictures regardless of content category (tobacco, pleasant, and unpleasant). Smokers in the Abstinent-High ΔQSU2 subgroup had both the largest increase in QSU2 scores and the largest [emotional – neutral] LPP scores. This pattern of results supports the hypothesis of negative reinforcement models that tobacco abstinence produces affective changes (as reflected in physiological responses to emotional stimuli) and that escaping or avoiding these affective changes is a powerful motivator of smoking behavior.

Potential limitations and future directions

A limitation of this study is that some of the peripheral psychophysiological measures did not replicate well-established effects. The expected main effect of picture category on zygomatic EMG (with greater responses to pleasant pictures) was not

found. Also, [pleasant – neutral] and [unpleasant – neutral] difference scores for orbicularis EMG and [pleasant – neutral] difference scores for SCR were not statistically significant, which fails to replicate the well-established relationship between picture arousal and these responses. For zygomatic and orbicularis EMG, this may have been due to the loss of data from several participants as a result of excessive EMG artifact. In fact, the typical pattern of increased orbicularis EMG during pleasant and unpleasant pictures, compared to neutral pictures, was evident in the nonsmokers and nonabstinent smokers, which suggests that the main effect of picture category (or group x picture category interaction) may have reached significance with fewer rejected trials. The lack of the typical SCR increase during pleasant pictures is more troubling, as no data were lost as a result of artifact. Based on these observations, the peripheral psychophysiological measures should be interpreted more cautiously than the LPP results. However, it should be noted that zygomatic EMG to tobacco pictures was greater in nonabstinent smokers than in nonsmokers, replicating the findings of a previous study (Geier et al., 2000) and that orbicularis EMG (often interpreted as a measure of attention; Bradley et al., 1990) was elevated in response to tobacco pictures in both nonabstinent and abstinent smokers, buttressing the hypothesis that tobacco cues are more motivationally relevant to smokers than nonsmokers.

A second limitation to this study is that the participants in this study were lighter smokers than those used in the majority of smoking studies. This raises several concerns, including those related to the idea that the smokers may not have been truly "dependent" on nicotine (cf. Heatherton et al., 1991) and that the abstinence effects

observed here may not be attributable to a "true" nicotine withdrawal episode. Both of these concerns make it difficult to generalize the conclusions drawn from this sample to clinical populations of heavy smokers who are trying to quit. However, this concern is somewhat mitigated by the fact that the studies mentioned above have reported similar effects in heavier smokers. Also, results from several studies suggest that craving, withdrawal symptoms, difficulty quitting, and signs of nicotine dependence are evident in even the lightest (Davies et al., 2000; O'Loughlin et al., 2003; Sayette et al., 2001) and most inexperienced (DiFranza et al., 2000; Kassel et al., 2007) smokers. Given the vulnerability of adolescent (Chassin et al., 1996) and college-age (Wetter et al., 2004) smokers to transition from light to heavy smoking, it is important to continue to study cue reactivity in these populations.

The overall pattern of results from this study indicates that smoking reduces emotional responses to motivationally relevant stimuli. This pattern adds to a growing body of evidence that smoking reduces negative affect and thus supports negative reinforcement models of tobacco use. The LPP results from this study suggest that smoking reduces negative affect through the reduction of selective attention to motivationally relevant stimuli. These data encourage further research into the neurobiological basis of these attentional processes and into new smoking cessation therapies that reduce smokers' attentional bias to emotional stimuli.

CHAPTER 5: EMOTIONAL REACTIVITY TO EMOTIONAL AND SMOKING CUES DURING TOBACCO ABSTINENCE: A BOLD FMRI STUDY

Upon cessation of smoking, the negative affective consequences, such as anxiety and cigarette craving, peak within 48 h and are thought to contribute critically to relapse (Hughes, 1992, 2007). The neural basis of abstinence-related emotional responses has been studied using blood-oxygenation-level-dependent functional magnetic resonance imaging (BOLD fMRI). These studies have generally used a smoking cue-reactivity paradigm in which the BOLD response is compared between smoking cues that are presumed to elicit cigarette craving and neutral control cues. Smoking cues produce increased activation in brain regions that have been implicated in the expression of fear and anxiety (e.g., amygdala; Due, Huettel, Hall, & Rubin, 2002), the rewarding effects of drugs of abuse (e.g., ventral striatum, anterior cingulate, and medial prefrontal cortex; David et al., 2005; Due et al., 2002; Franklin et al., 2007), and attention to emotionally evocative stimuli (e.g., posterior parietal areas; Due et al., 2002; Janes et al., 2009; McClernon et al., 2009). Recently, the dorsal striatum (caudate and putamen), an area implicated in craving and habitual drug use (Koob & Volkow, 2010; Volkow et al., 2006), has also been shown to have increased BOLD responses to tobacco cues in abstinent smokers (McClernon et al., 2009).

The involvement of these brain regions in smoking cue reactivity supports affective theories of tobacco addiction, which predict that smoking cues presented during abstinence elicit strong emotional responses and that escape from or avoidance of these affective states reinforces cigarette smoking (Baker et al., 2004; Koob & Le

Moal, 2001, 2008; Poulos et al., 1981; Solomon & Corbit, 1973; Watkins, Koob et al., 2000). Importantly, however, the tobacco cues differed from the neutral control cues in these studies on dimensions of affective valence (unpleasant vs. pleasant) and arousal (calm vs. exciting) in addition to smoking-related content and degree of craving induction. Valence and arousal have been shown to influence BOLD responses in many of the brain regions that have been implicated in tobacco cue reactivity (Bradley et al., 2003; Lang, Bradley, Fitzsimmons et al., 1998; Sabatinelli, Bradley, Fitzsimmons, & Lang, 2005; Sabatinelli, Bradley, Lang, Costa, & Versace, 2007; Sabatinelli, Lang et al., 2007). This makes it difficult to determine whether the smoking-related emotional responses observed in various brain regions were specific to tobacco cues, or whether they were due to nonspecific effects of smoking on reactivity to a broad range of emotional stimuli.

To address this issue, we have examined smokers' emotional responses to highly arousing pleasant and unpleasant cues in addition to tobacco cues and low-arousal, neutral controls (Chapters 3 and 4). These studies have shown that tobacco abstinence increases emotional reactivity to unpleasant cues as well as to tobacco cues, as indexed by suppression of the P300 (P3) event-related potential (ERP) to startle probes delivered during the cues. We have also found that smoking a cigarette reduces reactivity to unpleasant cues, as indexed by reduced fear-potentiated startle, and to tobacco, pleasant, and unpleasant cues, as indexed by late-positive ERPs to cue onset (Chapters 3 and 4). This set of results suggests that abstinence-induced changes in emotional responses are not limited to smoking-specific cues, but rather are the result of changes in brain

motivational systems that influence the overall level of reactivity to a broad spectrum of highly arousing stimuli. In this study, we used the same paradigm to disambiguate BOLD signals in response to smoking-specific cues from BOLD signals in response to arousing pleasant and unpleasant stimuli in general.

These signals were assessed in three groups of participants: nonsmokers, nonabstinent smokers, and smokers who were 24 h into a 48-h abstinence period. In previous studies, smoking cues elicited significant BOLD responses during smoking abstinence in the ventral striatum and other regions that contribute to appetitive emotional responses (e.g., David et al., 2005; Due et al., 2002; Franklin et al., 2007; Stippekohl et al., 2010). In contrast to the conditions in the current study, however, the experiments were conducted at the end of a period of abstinence. Using startle-probe P3 suppression, we have shown that when the prospect of smoking is not imminent, responses to smoking cues resemble those to unpleasant pictures (Chapter 3). Thus, we expected that BOLD responses measured during the middle of an abstinence period would closely resemble those to unpleasant pictures.

In this manuscript, we present full-brain, voxelwise analysis of BOLD cue reactivity to the emotional pictures in terms of two between-groups comparisons: nonabstinent smokers vs. nonsmokers and abstinent smokers vs. nonabstinent smokers. There was little evidence of significant differences in cue-induced BOLD responses between nonsmokers and nonabstinent smokers, but there were several areas where activation was significantly larger in abstinent smokers than nonabstinent smokers. For tobacco cues, these regions included several locations in the frontal cortex that have

been previously implicated addictive behavior. For both tobacco and unpleasant cues, there was significant abstinence-induced BOLD cue reactivity in the dorsal striatum and anterior cingulate, which further supports the role of these structures in smokers' affective responses.

Method

Participants

Eighteen daily smokers and nonsmokers were recruited from the university campus and surrounding community. Inclusion criteria for smokers were a history of smoking at least 5 cigarettes per day for at least 6 months, a baseline expired-air carbon monoxide (CO) level of at least 7 ppm (Marrone et al., 2010), willingness to abstain from smoking for 48 h if assigned to the abstinence condition, and no current use of over-the-counter or prescription stop-smoking remedies or current participation in smoking cessation counseling. Inclusion criteria for nonsmokers were a lifetime consumption of fewer than 20 cigarettes, no use of products containing nicotine or tobacco for at least 1 year, and a baseline expired-air CO level below 6 ppm. Exclusion criteria for both smokers and nonsmokers included: younger than 18 or older than 35 years old, uncorrected vision or hearing problems, current diagnosis of major psychiatric illness, claustrophobia, or medical conditions or use of medications that would interfere with the individual's ability to attend to the stimuli or that would be unsafe in the MR scanning environment. One female assigned to the abstinence condition dropped out of the study prior to the fMRI session, and another was excused because she failed to maintain abstinence prior to the fMRI session on two occasions.

This resulted in a final sample size of 16: 5 nonsmokers (4 female), 5 nonabstinent smokers (1 female), and 6 abstinent smokers (3 female).

All participants provided written informed consent to participate in the study and all procedures were approved by the University of Minnesota's Institutional Review Board. Participants had a choice of $50 or extra credit for their psychology course as compensation for completing the entire study. Smokers assigned to the abstinent group who met criteria for abstinence verification received an additional cash bonus of $25 or additional course credit.

Stimuli

Sixty full-color, 1024 x 768 pixel stimuli were presented using a 41 x 31 cm LCD video monitor (2190UXi, NEC, Itasca, IL) positioned at the rear of the scanner suite and were made visible to the subject via an adjustable mirror such that they subtended a viewing angle of 8 degrees vertically and 11 degrees horizontally. The stimuli were classified according to four categories: tobacco, pleasant, neutral, and unpleasant. The pleasant, neutral, and unpleasant stimuli were selected from the International Affective Picture System (Lang et al., 1999) and the tobacco stimuli were selected from a set of pictures collected by the authors from internet searches and print media and from a set provided by the lab of Paul Cinciripini (Carter et al., 2006). All

stimuli used in this study[5] were previously normed on measures of affective valence, arousal, and dominance in our laboratory (Engelmann & Cuthbert, 2008).

fMRI Acquisition

MRI images were acquired using a Siemens (Erlangen, Germany) TIM Trio 3T MR scanner at the University of Minnesota's Center for Magnetic Resonance Research running Syngo (vb15) software with full-body gradients (Avanto; 45 mT/m, 200 mT/m/ms slew), a 12-channel head-only receive coil and a full-body transmit coil. During the picture-viewing task, BOLD signal was measured using a T2*-weighted, gradient echo, echo-planar (EPI) pulse sequence with the following parameters: TR = 2000 ms, TE = 28 ms (Gorno-Tempini et al., 2002), flip angle = 81 degrees (Ernst, Bodenhausen, & Wokaun, 1987), matrix = 64 x 64, in-plane field-of-view = 192 mm, in-plane voxel size = 3.0 mm x 3.0 mm, echo spacing = 0.47 ms, receive bandwidth = 2442 Hz/pixel, phase-encode direction = anterior-to-posterior. Thirty-five axial-oblique slices were oriented in a plane parallel to the ventral surface of the prefrontal cortex, and acquired in an interleaved order with a thickness of 2 mm and a between-slice gap of 1 mm. This slice geometry was used to minimize signal loss due to susceptibility artifact caused by the sinuses (Deichmann, Gottfried, Hutton, & Turner, 2003), and to maximize the brain volume imaged.

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[5] The IAPS pictures used were: Pleasant: 4606, 4611, 4651, 4653, 4687, 4694, 7330, 7410, 7450, 7470, 7488, 8034, 8117, 8499, 8502; Neutral: 2383, 2516, 2880, 2980, 5531, 5740, 7050, 7080, 7095, 7170, 7175, 7179, 7205, 7550, 7705; Unpleasant: 1051, 1270, 1300, 1930, 2095, 6022, 6200, 6243, 6244, 6260, 8480, 9042, 9290, 8342, 9592. The tobacco stimuli from Carter et al. (2006) were: 402, 403, and 414. The remaining smoking stimuli were from Engelmann and Cuthbert (2008): S254, S359, S369, S377, S413, S435, S441, S454, S457, S460, S466, and S479. These stimuli are available upon request from the author.

For purposes of mapping the BOLD signal to specific anatomical regions, a 3-dimensional, high-resolution (1 mm^3 voxels), T1-weighted image was obtained using an inversion-recovery pulse sequence (TR = 2600 ms, TE = 3.02 ms, TI = 900 ms, flip angle = 8 degrees). A dual gradient echo magnetic field map was obtained and used to correct geometric distortions in the EPI images. This scan used the same slice orientation and voxel size as the EPI scans, with the following parameters: TR = 488 ms, TE_1 = 5.48 ms, TE_2 = 7.94 ms, flip angle = 60 degrees.

Procedure

Participants came to the laboratory for three sessions. During Visit 1, participants completed several questionnaire measures to assess smoking history, fear and anxiety, and cigarette withdrawal symptoms and craving (see Table 2-1). All participants also provided breath samples for baseline expired-air CO measurement. CO level was defined as the mean of two samples taken using a PiCO Smokerlyzer (Bedfont Scientific USA, Williamsburg, VA). At the end of Visit 1, smokers were informed of their random assignment to the nonabstinent or abstinent condition, and were instructed, respectively, to smoke as normal or to abstain from cigarettes and all other products containing nicotine or tobacco beginning 24 h prior to the start of Visit 2, through the end of Visit 3. Visits 2 and 3 were scheduled 24 h apart, within 3 weeks of Visit 1.

Upon arrival at the laboratory for Visit 2, smokers in the nonabstinent group were asked to completely smoke one of their regular cigarettes, and smoking status was verified using expired-air CO. Smokers assigned to the abstinent condition who

reported no cigarette consumption 24 h prior to the start of the visit and who had CO levels of less than 7 ppm or half of their baseline value (whichever was lower) were classified as successfully abstinent (e.g., Marrone et al., 2010). All participants then completed a brief set of questionnaires that measured state anxiety, cigarette craving, and withdrawal symptoms.

After completing the questionnaires, the participant was prepared for the MR scanning procedure and positioned at the isocenter of the magnetic field. After a brief acclimation period, the picture-viewing task began. This consisted of a short practice block (4 picture trials, 1 from each category), followed by four full picture-viewing blocks, each consisting of 15 picture trials, presented in a random order with respect to picture category. A total of four picture orders were used, distributed randomly across subjects to reduce the likelihood that order effects would influence the results. At the start of each block, the participant was instructed to pay attention to the pictures and reminded to hold as still as possible to reduce artifacts in the MRI images. EPI images were acquired continuously from the beginning to the end of each block (scan length = 5.5 min). On each trial, a picture was presented for 6 s, followed by an intertrial interval (ITI) ranging from 12-18 s, during which a gray screen was presented. The magnetic field map (scan length = 2 min) was acquired between the second and third picture-viewing block and the high-resolution anatomical image (scan length = 8 min) was acquired after the final block. At the conclusion of the anatomical scan, the participant was removed from the scanner, asked to complete a brief questionnaire, and dismissed until Visit 3.

The beginning of Visit 3 used the same procedure as the beginning of Visit 2 (i.e., nonabstinent smokers smoke, CO measurement, and questionnaires). All participants then completed two picture memory tasks, the results of which will not be reported here, followed by a subjective ratings task. During the subjective ratings task, participants viewed each picture that was presented during the fMRI session and used a computerized visual analog scale with five anchor points to rate the picture on the dimensions of affective valence and arousal (Hodes et al., 1985). Participants were then debriefed, compensated, and dismissed.

Statistical Analysis of Self-Report Data

Questionnaire scores and self-reported valence and arousal were analyzed using mixed model ANOVAs (SYSTAT 12 Software, Chicago, IL) with group as a between subjects factor and visit or picture category as within subjects factors. Significant two-way interactions were followed up with one-way ANOVAs for group within each visit or picture category, followed by Tukey's Honestly Significant Difference (HSD) *post hoc* tests for comparison of individual group means. The picture category factor consisted of three levels: [tobacco – neutral], [pleasant – neutral], and [unpleasant – neutral] difference scores (e.g., Versace et al., 2010). In all cases, the significance criterion was $p < .05$ and multivariate tests (Wilks λ and its approximate F statistic) were used for effects involving repeated measures (Johnson & Wichern, 2002).

Statistical Analysis of fMRI Data

fMRI analyses were conducted using SPM8 (Wellcome Trust Centre for Neuroimaging, London). Each subject's raw fMRI time course was corrected for slice

acquisition time, followed by spatial realignment using rigid body transformations to correct for head motion and field map-based estimates of voxel displacement to correct for geometric distortions in the EPI images. Next, individual participants' fMRI scans were co-registered to their anatomical scan. The anatomical scan was segmented by tissue type (gray matter, white matter, cerebrospinal fluid) and the segmentation parameters were used to warp the fMRI and structural data into standard stereotaxic space (Montreal Neurological Institute) with 3-mm^3 and 1-mm^3 voxel sizes, respectively, followed by spatial smoothing of the fMRI data with a with a Gaussian filter (5-mm^3 full with at half maximum).

After preprocessing, the fMRI time course from each within-brain voxel of each subject's data was entered into a first-order, fixed-effects general linear model (Friston et al., 1995). This model consisted of a set of three explanatory variables per picture category that were created by convolving a boxcar function containing stimulus timing information with a canonical hemodynamic response function and its time and dispersion derivatives. The movement parameters (translation and rotation) from spatial realignment were included in the model as nuisance variables, and a constant term was also included. The time courses of the explanatory variables and fMRI data were high-pass-filtered with a time constant of 50 s to reduce the impact of low-frequency drift in the MR signal on the model fit. Parameters for each explanatory variable were estimated using a restricted maximum likelihood model with an autoregressive AR(1) term to account for temporal correlation in the residuals. The parameter estimates for the canonical hemodynamic response function were used to

create the following contrast images for each subject: [picture > baseline] for tobacco, pleasant, neutral, and unpleasant pictures and [emotional > neutral] for tobacco, pleasant, and unpleasant pictures.

Group analyses were conducted on the contrast images from the first-order analysis using random effects general linear models (Holmes & Friston, 1998). A preliminary analysis of the [picture > baseline] contrasts for all 16 subjects was conducted using one-sample *t* tests. The purpose of this analysis was to verify that the pictures produced robust activity in regions that have previously been shown to be responsive to emotional pictures. The main analyses were designed to identify brain regions where emotional reactivity varied as a function of smoking status. These analyses were conducted on the [emotional > neutral] contrast images for tobacco, pleasant, and unpleasant pictures, and consisted of two contrasts: [nonabstinent > nonsmoker] and [abstinent > nonabstinent] (e.g., Hogle & Curtin, 2006). The significance of these contrasts was evaluated using two-sample *t* tests. Activations were considered significant with an uncorrected $T \geq 3.0$ and a minimum cluster size of 10 contiguous voxels, which controlled the type I error rate at approximately $p < .05$ (Forman et al., 1995; Lieberman & Cunningham, 2009; Xiong, Gao, Lancaster, & Fox, 1995). The *T* statistics for significant voxels were overlaid onto an anatomical reference image that was obtained by averaging the spatially normalized anatomical images across all 16 subjects. Anatomical labels for significant clusters were obtained by entering the coordinates into a computerized Talairach atlas (Lancaster et al., 1997; Lancaster et al., 2000) and were verified by visual inspection.

Results

Participant demographics and smoking history

Participants were adults in their early twenties, and smokers were considered light to moderate smokers according to the number of cigarettes smoked per day, CO levels produced during Visit 1, and scores on the FTND and HSI (Table 5-1). Nonabstinent and abstinent smokers did not differ significantly on the number of cigarettes smoked per day, maximum daily smoking rate, number of years they have been smoking, number of serious quit attempts, longest duration of abstinence during a quit attempt, FTND score, or HSI score (see Table 5-1 for *F* tests).

Abstinence verification

There was a significant group x category interaction for expired-air CO scores [$F(4,18) = 3.51$, $p < .05$, $\lambda = .32$]. This interaction was due to larger CO scores in abstinent and nonabstinent smokers than in nonsmokers during Visit 1, and larger CO scores in nonabstinent smokers than in abstinent smokers or nonsmokers during Visits 2 and 3 (Table 5-1). This pattern of CO scores is consistent with smoking prior to Visits 2 and 3 in the nonabstinent group and with abstinence prior to Visits 2 and 3 in the abstinent group. Self-reported abstinence agreed with expired-air CO scores in all participants.

Table 5-1. Participant Demographics, Smoking History, and Questionnaire Scores (Mean ± SEM)

	Session	Nonsmokers	Nonabstinent Smokers	Abstinent Smokers	Group ANOVA
N (female)		5 (4)	5 (1)	6 (3)	
N minority		3	1	2	
Age (years)		20.3 ± 0.6	24.6 ± 2.4	20.9 ± 0.6	$F = 2.8$
Current CPD			10.8 ± 2.4	10.7 ± 0.6	$F < 1$
Maximum CPD			11.8 ± 2.5	18.5 ± 4.6	$F = 1.4$
Years Smoked			9.5 ± 2.7	4.0 ± 1.8	$F = 3.1$
Quit Attempts			1.8 ± 1.2	0.5 ± 0.3	$F = 1.5$
Max days abstinent			10.3 ± 8.9	257.5 ± 197.5	$F = 2.8$
FTND	1		2.8 ± 0.8	3.4 ± 0.6	$F < 1$
HSI	1		2.0 ± 0.4	2.1 ± 0.4	$F < 1$
FSS	1	1.8 ± 0.3	1.7 ± 0.2	1.9 ± 0.2	$F < 1$
EASI-Sociability	1	3.3 ± 0.4	2.9 ± 0.4	3.2 ± 0.4	$F < 1$
EASI-Activity	1	2.6 ± 0.2^{a}	1.4 ± 0.2^{b}	$1.8 \pm 0.3^{a,b}$	$F = 6.1$*
EASI-Impulsivity	1	2.2 ± 0.1	2.4 ± 0.5	2.4 ± 0.2	$F < 1$
EASI-Fear	1	2.6 ± 0.4	1.8 ± 0.2	2.4 ± 0.4	$F = 1.05$
EASI-Anxiety	1	2.3 ± 0.4	2.2 ± 0.3	2.3 ± 0.3	$F < 1$
STAI-Trait	1	2.2 ± 0.2	1.7 ± 0.1	1.9 ± 0.2	$F = 1.4$
STAI-State	1	1.9 ± 0.2	1.5 ± 0.1	1.4 ± 0.1	$F = 2.2$
	2	1.7 ± 0.3	1.6 ± 0.2	1.8 ± 0.2	$F < 1$
	3	1.9 ± 0.1^{a}	1.3 ± 0.1^{b}	1.9 ± 0.2^{a}	$F = 4.1$*
MNWS	1		0.7 ± 0.2	0.8 ± 0.4	$F < 1$
	2		0.4 ± 0.1	1.2 ± 0.2	$F = 6.9$*
	3		0.4 ± 0.3	1.0 ± 0.3	$F = 2.2$
QSU Factor 1	1		3.8 ± 0.4	3.7 ± 0.2	$F < 1$
	2		3.1 ± 0.4	4.5 ± 0.2	$F = 11.4$**
	3		3.0 ± 0.3	4.3 ± 0.3	$F = 10.5$**
QSU Factor 2	1		2.3 ± 0.6	2.8 ± 0.2	$F < 1$
	2		2.5 ± 0.5	3.8 ± 0.3	$F = 5.7$*
	3		2.6 ± 0.5	3.9 ± 0.4	$F = 4.3$†
Expired-air CO	1	2.0 ± 0.6	18.1 ± 6.7	9.6 ± 1.8	$F = 2.8$
	2	1.5 ± 0.3^{a}	23.6 ± 5.5^{b}	2.3 ± 0.5^{a}	$F = 13.4$***
	3	1.9 ± 0.8^{a}	18.2 ± 4.1^{b}	2.9 ± 0.4^{a}	$F = 15.4$***

Note: CPD = cigarettes per day, FTND = Fagerström Test of Nicotine Dependence (Heatherton et al., 1991), HSI = Heavy Smoking Index (Heatherton et al., 1991), FSS = Fear Survey Schedule (Wolpe & Lang, 1964), EASI = Emotionality, Activity, Sociability, and Impulsivity Inventory (Buss & Plomin, 1975, 1984), STAI = State-Trait Anxiety Inventory (Spielberger, 1979), MNWS = Minnesota Nicotine Withdrawal Scale (Hughes & Hatsukami, 1998), QSU = Questionnaire of Smoking Urges (Tiffany & Drobes, 1991), where Factor 1 = craving for the appetitive consequences of smoking and Factor 2 = desire to smoke to avoid withdrawal symptoms, CO = carbon monoxide. The questionnaire scores were measured on the following scales: FTND = 1-10, HSI = 1-6, FSS and EASI = 1-5, STAI = 1-4, MNWS = 0-4, QSU = 1-7. *Asterisks* indicate statistically significant *F* tests in a one-way ANOVA for group (* $p < .05$, ** $p < .01$, *** $p < .001$) and the *dagger* represents an *F* test that approached statistical significance († $p < .1$). On measures for which a statistically significant ANOVA compared all three groups, means that do not share superscripts significantly differed in Tukey HSD *post hoc* tests.

Self-report measures

Nonsmokers scored significantly higher than nonabstinent smokers, but not abstinent smokers, on the Activity scale of the EASI. The three groups did not significantly differ on any other questionnaire measure during Visit 1 (Table 5-1). For state anxiety, there was a significant group x visit interaction [$F(4,24) = 3.34$, $p < .05$, $\lambda = .41$]: During Visits 1 and 2, there were no significant differences between groups in state anxiety scores, but during Visit 3, abstinent smokers scored higher than nonsmokers (Table 5-1). The group x visit interactions for MNWS, QSU1, and QSU2 scores did not approach statistical significance [all Fs < 2.9, all ps > .1, all λs > .5], but the main effect of group was significant for the QSU1 [$F(1,9) = 12.82$, $p < .001$] and approached significance for the QSU2 [$F(1,9) = 3.64$, $p = .09$], but not the MNWS [$F(1,8) < 1$, $p > .1$]. Follow-up tests indicated that MNWS, QSU1, and QSU2 scores were significantly higher in abstinent smokers than nonabstinent smokers during Visit 2, and that this difference remained significant during Visit 3 for QSU1 scores and approached significance for QSU2 scores (Table 5-1).

Subjective ratings of the pictures

Valence and arousal ratings are presented in Figure 5-1. For valence, there was a significant group x category interaction for [emotional – neutral] difference scores [$F(4,24) = 6.94$, $p < .01$, $\lambda = .22$], which was the result of a significant main effect of group for [tobacco – neutral] scores [$F(2,13) = 26.15$, $p < .001$], but not for [pleasant – neutral] [$F(2,13) = 2.95$, $p = .09$] or [unpleasant – neutral] [$F(2,13) < 1$, $p > .1$] scores. Tukey *post hoc* tests indicated that all three groups significantly differed in their

[tobacco – neutral] difference scores, with abstinent smokers rating the tobacco cues as most pleasant, followed by nonabstinent smokers and nonsmokers. For arousal, the group x category interaction [$F(4,24) = 1.84$, $p > .1$, $\lambda = .59$] and group main effect [$F(2,13) = 1.47$, $p > .1$] did not approach significance, but the main effect of picture category was significant [$F(2,12) = 36.99$, $p < .001$, $\lambda = .14$]. This is reflective of the overall higher [emotional – neutral] scores for pleasant and unpleasant pictures, compared to tobacco pictures.

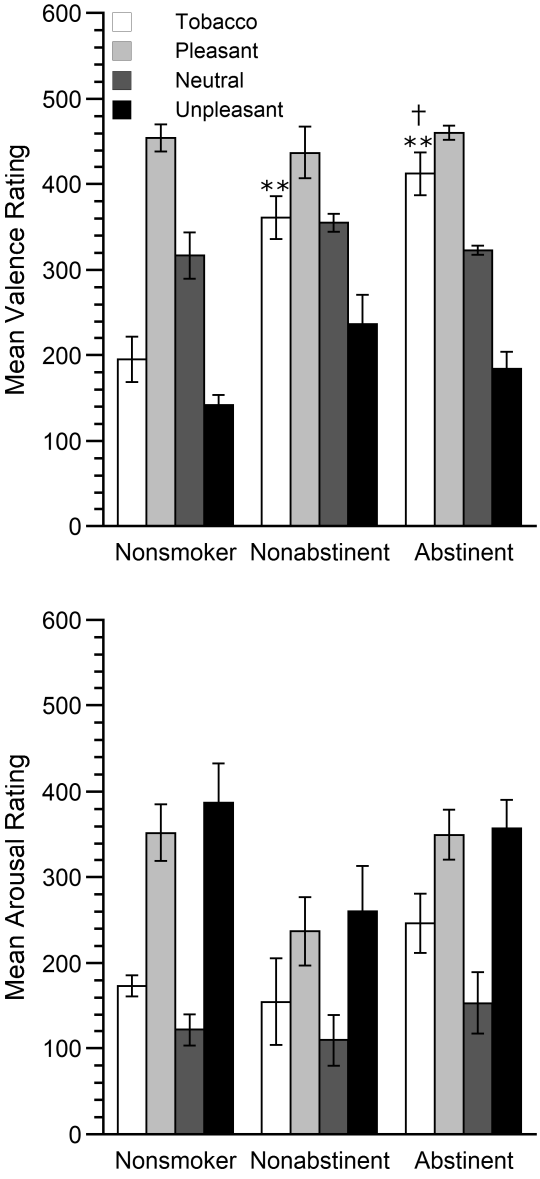

Figure 5-1. Valence (*upper panel*) and arousal (*lower panel*) ratings of the pictures from each category during the subjective ratings task. The ratings were obtained via a computerized visual analog scale, and the values on the *y* axis represent the mean coordinate along that scale. Error bars depict the mean ± 1 *SEM*. *n*s = 5, 5, and 6 for nonsmokers, nonabstinent smokers, and abstinent smokers, respectively. ** Indicates that the mean [emotional – neutral] difference score for the marked group and picture category was significantly different from the same score in the nonsmoker group, as determined by Tukey HSD *post hoc* tests ($p < .01$). † Indicates that the mean [emotional – neutral] difference score for the marked group and picture category was significantly different from the same score in the nonabstinent group, as determined by Tukey HSD *post hoc* tests ($p < .05$).

Verification of BOLD responses to all picture categories

The results for the one sample *t* tests for all participants' [picture > baseline] contrasts are presented in Figure 5-2. Visual inspection of the data revealed a large cluster of significant activation, with the largest *T* statistics located in the occipital lobe. There were larger maximum *T* statistics and volumes of activation for pleasant and unpleasant pictures than for neutral pictures, which replicates the findings from several previous studies (Bradley et al., 2003; Lang, Bradley, Fitzsimmons et al., 1998; Sabatinelli, Lang et al., 2007). Total volumes of activation for tobacco, pleasant, neutral, and unpleasant pictures, respectively, were 233.982, 480.087, 384.534, and 528.255 cm^3. The maximum $T(15)$ statistics were 14.84, 15.62, 10.10, and 13.23, and uncorrected *p* values for significant voxels were ≤ .003.

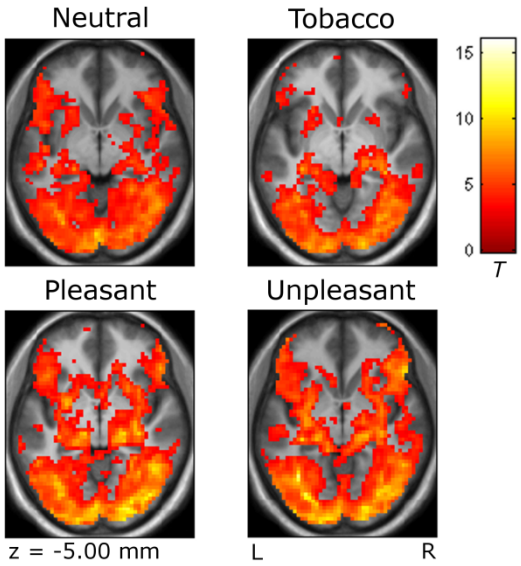

Figure 5-2. Statistical parametric maps of significant [picture > baseline] BOLD activation for tobacco, pleasant, neutral, and unpleasant pictures, averaged across all subjects and overlaid onto a mean structural image. *Colored pixels* indicate that the voxel exceeded the significance criterion of $T(16) \geq 3$ within a cluster of at least 10 contiguous voxels exceeding this threshold. $n = 16$. *L* = left, *R* = right, *z* = the location of all images along the inferior-superior axis in Talairach space.

BOLD responses: Tobacco > Neutral

Clusters where the [tobacco > neutral] contrast was significantly greater in nonabstinent smokers than in nonsmokers ([nonabstinent > nonsmoker]) and in abstinent smokers than in nonabstinent smokers ([abstinent > nonabstinent]) are presented in Table 5-2. These contrasts revealed that nonsmokers' and nonabstinent smokers' BOLD cue reactivity did not differ significantly, with the exception of a small region of posterior frontal white matter in the right hemisphere. However, BOLD cue reactivity was significantly greater in abstinent smokers than in nonabstinent smokers in several areas (Figure 5-3). These included a volume that enclosed parts of the anterior cingulate cortex and dorsal striatum, bilateral volumes in the paracentral lobule, posterior cingulate, and middle temporal gyrus, right hemisphere volumes in the hippocampus, inferior parietal lobule, and precentral, medial, and inferior frontal gyri, and left hemisphere volumes in the fusiform, superior temporal, and superior and middle frontal gyri.

Table 5-2. Brain areas of significant activation where the [tobacco > neutral] contrast was greater in nonabstinent smokers than in nonsmokers or greater in abstinent smokers than in nonabstinent smokers

			Talairach Coordinates (mm)			Cluster size	
Brain Area	Side	BA	x	y	z	(mm^3)	T_{max}
[Nonabstinent > Nonsmoker]							
White matter	R		21	-7	34	270	4.78
[Abstinent > Nonabstinent]							
Frontal cortex							
Superior frontal gyrus	L	10	-6	59	-2	297	3.71
Middle frontal gyrus	L	10	-42	47	19	1539	6.77
	L	8	-21	29	40	405	6.26
Inferior frontal gyrus	R	46	45	38	7	351	4.17
	R	45	48	32	4	351	4.05
Medial frontal gyrus	R	11	9	26	-14	351	4.32
Precentral gyrus	R	9	39	11	37	351	4.78
Paracentral lobule	L/R	5/6	-3	-40	58	1215	4.44
Temporal cortex							
Superior temporal gyrus	L	22	-60	-40	16	351	4.31
Middle temporal gyrus	L	39	-42	-70	16	1755	14.89
	R	37	48	-49	-5	432	6.05
	R	39	45	-67	16	351	4.60
Fusiform gyrus	L	37	-36	-46	-11	378	7.64
Hippocampus	R		33	-34	-5	540	5.16
Parietal cortex							
Inferior parietal lobule	R	40	63	-25	25	567	4.66
Cingulate and Subcortical Areas							
Anterior Cingulate/Caudate	R	25	3	17	-5	1350	4.45
Posterior Cingulate	L/R	24	0	13	25	432	4.44

Note: R = right hemisphere, L = left hemisphere, L/R = the cluster was along the midline and included regions in both hemispheres, BA = Brodmann area. Talairach coordinates are expressed as follows, with the origin at the anterior commissure: *x* increases from left to right, *y* increases from posterior to anterior, and *z* increases from inferior to superior. T_{max} = the maximum *T* statistic found in the cluster, with 8 degrees of freedom for the [nonabstinent > nonsmoker] contrast and 9 degrees of freedom for the [abstinent > nonabstinent contrast]. The significance criterion was $T \geq 3$ with a minimum cluster size of 10 contiguous voxels (270 mm^3). For T_{max}, all uncorrected *p*s $\leq .002$.

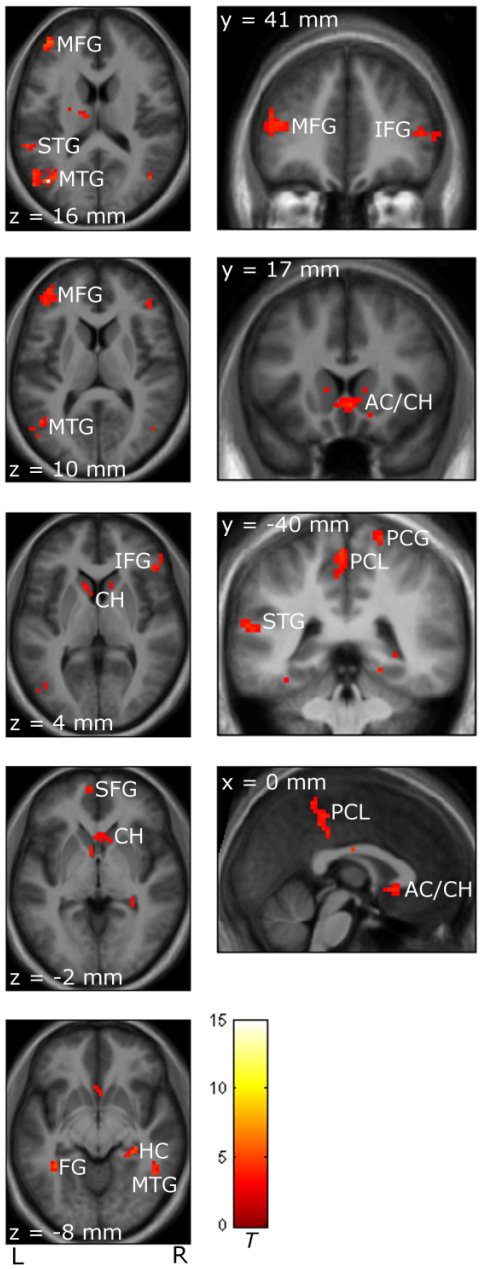

Figure 5-3. Statistical parametric maps of clusters where the [tobacco > neutral] contrast was greater in abstinent smokers than in nonabstinent smokers, overlaid onto a mean structural image. *Colored pixels* indicate that the voxel exceeded the significance criterion of $T(9) \geq 3$ within a cluster of at least 10 contiguous voxels exceeding this threshold. *L* = left, *R* = right, *x*, *y*, *z* = the location of each slice along the left-right, posterior-anterior, and inferior-superior axes of Talairach space, respectively. *AC* = anterior cingulate cortex, *CH* = head of the caudate nucleus, *FG* = fusiform gyrus, *HC* = hippocampus, *IFG* = inferior frontal gyrus, *MFG* = middle frontal gyrus, *MTG* = middle temporal gyrus, *PCG* = precentral gyrus, *PCL* = paracentral lobule, *SFG* = superior frontal gyrus, *STG* = superior temporal gyrus.

BOLD responses: Pleasant > Neutral

In the [nonabstinent > nonsmoker] comparison of the [pleasant > neutral] contrast, there were no significant clusters. In the [abstinent > nonabstinent] comparison, there were significant clusters in the right angular gyrus and precuneus and in the left lingual gyrus (Table 5-3 and Figure 5-4, left column).

Table 5-3. Brain areas of significant activation where the [pleasant > neutral] contrast was greater in nonabstinent smokers than in nonsmokers or greater in abstinent smokers than in nonabstinent smokers

			Talairach Coordinates (mm)			Cluster size	
Brain Area	Side	BA	x	y	z	(mm^3)	T_{max}
[Nonabstinent > Nonsmoker]							
No significant clusters							
[Abstinent > Nonabstinent]							
Temporal cortex							
Angular gyrus	R	39	36	-73	31	432	6.09
Occipital cortex							
Lingual gyrus	L	18	-6	-82	-8	513	4.76
Precuneus	R	23	0	-55	10	270	3.71

Note: R = right hemisphere, L = left hemisphere, BA = Brodmann area. Talairach coordinates are expressed as follows, with the origin at the anterior commissure: *x* increases from left to right, *y* increases from posterior to anterior, and *z* increases from inferior to superior. T_{max} = the maximum *T* statistic found in the cluster, with 8 degrees of freedom for the [nonabstinent > nonsmoker] contrast and 9 degrees of freedom for the [abstinent > nonabstinent contrast]. The significance criterion was $T \geq 3$ with a minimum cluster size of 10 contiguous voxels (270 mm^3). For T_{max}, all uncorrected *p*s $\leq .002$.

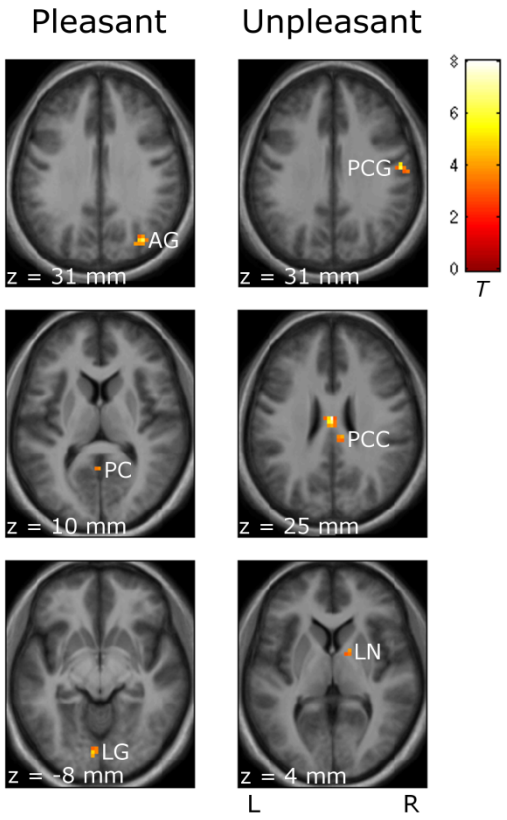

Figure 5-4. Statistical parametric maps of clusters where the [pleasant > neutral] (*left column*) or [unpleasant > neutral] (*right column*) contrasts were greater in abstinent smokers than in nonabstinent smokers, overlaid onto a mean structural image. *Colored pixels* indicate that the voxel exceeded the significance criterion of $T(9) \geq 3$ within a cluster of at least 10 contiguous voxels exceeding this threshold. *L* = left, *R* = right, *z* = the location of each slice along the inferior-superior axis of Talairach space. *AG* = angular gyrus, *LG* = lingual gyrus, *LN* = lentiform nucleus, *PC* = precuneus, *PCC* = posterior cingulate cortex, *PCG* = precentral gyrus.

BOLD responses: Unpleasant > Neutral

In the [nonabstinent > nonsmoker] comparison of the [unpleasant > neutral] contrast, there were no significant clusters. In the [abstinent > nonabstinent] comparison, there were significant clusters bilaterally in the posterior cingulate, and in the right hemisphere in the precentral and postcentral gyri, anterior cingulate, and lentiform nucleus of the dorsal striatum (Table 5-4 and Figure 5-4; right column). Interestingly, the significant [abstinent > nonabstinent] cluster that enclosed parts of the

anterior cingulate and dorsal striatum for BOLD cue reactivity to unpleasant pictures was similar to a cluster seen in the same comparison for BOLD cue reactivity to tobacco pictures (Figure 5-5).

Table 5-4. Brain areas of significant activation where the [unpleasant > neutral] contrast was greater in nonabstinent smokers than in nonsmokers or greater in abstinent smokers than in nonabstinent smokers

			Talairach Coordinates (mm)			Cluster size	
Brain Area	Side	BA	x	y	z	(mm^3)	T_{max}
[Nonabstinent > Nonsmoker]							
No significant clusters							
[Abstinent > Nonabstinent]							
Frontal cortex							
Precentral gyrus	R	4	57	-13	31	351	5.97
Parietal cortex							
Postcentral gyrus	R	2	33	-28	37	297	3.38
Cingulate and Subcortical Areas							
Anterior Cingulate/Caudate	R	25	6	17	-5	351	5.17
Posterior Cingulate	L/R	23-4	0	-13	25	1296	7.64
Lentiform nucleus	R		15	2	4	405	3.85

Note: R = right hemisphere, L = left hemisphere, L/R = the cluster was along the midline and included regions in both hemispheres, BA = Brodmann area. Talairach coordinates are expressed as follows, with the origin at the anterior commissure: *x* increases from left to right, *y* increases from posterior to anterior, and *z* increases from inferior to superior. T_{max} = the maximum *T* statistic found in the cluster, with 8 degrees of freedom for the [nonabstinent > nonsmoker] contrast and 9 degrees of freedom for the [abstinent > nonabstinent contrast]. The significance criterion was $T \geq 3$ with a minimum cluster size of 10 contiguous voxels (270 mm^3). For T_{max}, all uncorrected ps $\leq .004$.

Discussion

This study compared BOLD cue reactivity to tobacco, pleasant, neutral, and unpleasant pictures in nonsmokers, nonabstinent smokers, and abstinent smokers who were 24 h into a 48-h deprivation period. There were two main findings. First, there were significant abstinence-induced BOLD responses to both tobacco and unpleasant cues in the dorsal striatum and anterior cingulate cortex. Second, the responses of many brain regions, primarily in the frontal and inferior temporal lobes, were specific to tobacco cues in abstinent smokers.

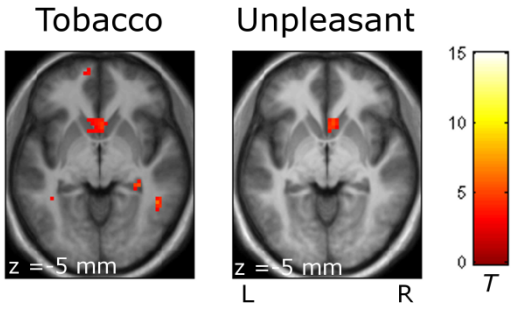

Figure 5-5. Similar areas of the anterior cingulate cortex and dorsal striatum have significantly greater BOLD responses in abstinent smokers than in nonabstinent smokers for the [tobacco > neutral] (*left panel*) and [unpleasant > neutral] (*right panel*) contrasts. *Colored pixels* indicate that the voxel exceeded the significance criterion of $T(9) \geq 3$ within a cluster of at least 10 contiguous voxels exceeding this threshold. *L* = left, *R* = right, *z* = the location of each slice along the inferior-superior axis of Talairach space.

Dorsal striatal responses to tobacco and unpleasant pictures

In this study, BOLD responses in the head of the caudate nucleus were significantly larger in abstinent smokers than in nonabstinent smokers for the [tobacco > neutral] contrast. Our results differ from most studies of smoking cue reactivity in that we found significant BOLD cue reactivity in the dorsal, rather than ventral, striatum. One other study has also found significant evidence of dorsal, but not ventral, striatal responses to smoking cues (McClernon et al., 2009). Because the dorsal striatum has been implicated in habit learning (e.g., Jog et al., 1999), the McClernon et al. (2009) finding has been interpreted in terms of the ability of tobacco cues to elicit "automatic" drug seeking behavior in experienced smokers. This interpretation arises from incentive-sensitization theories of addiction, which predict that as drug users become more experienced, control over drug seeking transitions from ventral to dorsal striatum (Everitt et al., 2008; Everitt & Robbins, 2005). However, several studies have found evidence of ventral, but not dorsal, striatal activation in response to smoking cues in participants with similar smoking histories to those in this study and in the McClernon

et al. (2009) study (e.g., David et al., 2007; David et al., 2005; Due et al., 2002; Franklin et al., 2007; Stippekohl et al., 2010). Thus, there may be an additional role for the dorsal striatum in addiction beyond the transition from initial to chronic drug use.

Importantly, studies that found significant ventral striatal cue reactivity found this effect in nonabstinent smokers (David et al., 2007; Franklin et al., 2007; Stippekohl et al., 2010) or at the end of a short (10-12 h) deprivation period, for which smoking was permitted immediately after the session (David et al., 2005; Due et al., 2002; Stippekohl et al., 2010). Both our study and McClernon et al. (2009) measured cue reactivity 24 h into a 48-h deprivation period, at which time dorsal striatal cue reactivity was evident in the abstinent group. Thus, a possible difference between the roles of the ventral and dorsal striatum, respectively, in cue reactivity may be in the response to cues under conditions of drug availability and unavailability. This hypothesis is also supported by two other findings. First, nonabstinent smokers who were told that they could not smoke after a test session had larger BOLD responses in the caudate to simulated monetary gains than did smokers who were told that they could smoke immediately after the session (Wilson, Sayette, Delgado, & Fiez, 2008). Second, cocaine users who had not used cocaine for 2-3 days showed an increase in dopamine release in the dorsal striatum in response to visual cocaine cues (Volkow et al., 2006). Although no explicit manipulations were made regarding the availability of cocaine after the cue reactivity session (Volkow et al., 2006), it is reasonable to assume that post-session cocaine availability was uncertain for most of the participants (i.e., cocaine is less readily available than cigarettes).

An important question remains as to the affective nature of dorsal striatal responses to smoking cues under conditions of drug unavailability: Are these responses appetitive, as they are presumed to be when mediated by the ventral striatum, or are they aversive, as would be predicted by negative reinforcement theories? One way of addressing the affective nature of dorsal striatum-mediated cue reactivity is to compare the [tobacco > neutral] BOLD contrast to the [unpleasant > neutral] and [pleasant > neutral] BOLD contrasts. In this comparison, we found abstinence-induced increases in [unpleasant > neutral] cue reactivity in similar regions of the dorsal striatum to those that showed significant [tobacco > neutral] cue reactivity (Figure 5-5). To our knowledge, this is the first study to demonstrate this effect, and it raises the intriguing possibility that emotional responses to tobacco and unpleasant cues share a common neurobiological mechanism under conditions of tobacco abstinence in which smoking is not imminently possible, and that this mechanism may include the dorsal striatum.

The possibility of dorsal striatal involvement in abstinent smokers' reactions to tobacco and unpleasant cues is consistent with a contemporary negative reinforcement model of tobacco addiction in which drug use is seen as "compulsive" in addicted individuals (Koob & Le Moal, 2001, 2008; Koob & Volkow, 2010). This model postulates that drug cues and stressors elicit an anxiety-like state that motivates drug seeking, similar to the motivating effects of anxiogenic obsessions on the compulsions seen in patients with obsessive-compulsive disorder (OCD). Studies of OCD patients have shown that obsessions and compulsions are associated with increased neural activity in the head of the caudate (Baxter et al., 1992; Guehl et al., 2008; Molina et al.,

1995), and that this effect may be specifically related to the anxiogenic characteristics of the obsessions and compulsions (Lucey et al., 1995). This is analogous to the presumably anxiogenic drug cravings elicited by tobacco and unpleasant cues that resulted in dorsal striatal activation in the current sample of abstinent smokers. Thus, the dorsal striatum may have an important role in the negative affective consequences of exposure to drug cues and in motivating escape from these consequences. Given the role of drug-related and unpleasant cues in relapse precipitation (e.g., Shiffman et al., 1996), it is important to further study the role of the dorsal striatum in smokers' emotional cue reactivity.

Anterior cingulate responses to tobacco and unpleasant pictures

We also found significantly greater [tobacco > neutral] and [unpleasant > neutral], but not [pleasant > neutral], cue reactivity in abstinent smokers than in nonabstinent smokers in the genual and subgenual areas of the anterior cingulate cortex. These areas have been implicated in cue-induced cigarette (Brody, Mandelkern et al., 2004; Brody et al., 2002; Brody, Olmstead et al., 2004; David et al., 2005; Due et al., 2002; Janes et al., 2009) and cocaine (Childress et al., 1999; Garavan et al., 2000; Maas et al., 1998) craving, which has lead to the hypothesis that the role of the anterior cingulate in addiction may be in the subjective experience of craving and in attempts to inhibit drug-taking behavior in response to craving (Goldstein & Volkow, 2002).

Negative reinforcement theories of addiction suggest that unpleasant cues elicit cigarette craving because of the individual smoker's past experience with the anxiolytic consequences of smoking a cigarette (e.g., Baker et al., 2004). Thus, our finding of

significant BOLD cue reactivity to unpleasant cues in the anterior cingulate may reflect activation of neural networks that prepare the individual to escape negative affect via smoking, which is consistent with the involvement of the anterior cingulate in escape and avoidance learning (e.g., Mobbs et al., 2009). Negative reinforcement theories also predict that tobacco cues are aversive, either via the elicitation of conditioned withdrawal symptoms (Poulos et al., 1981) or a state of frustrative non-reward (Drobes et al., 2001). Our finding that tobacco cues elicited significant BOLD cue reactivity in the anterior cingulate and our finding from a previous study that startle probe P3 responses to tobacco cues resembled those to unpleasant cues in abstinent smokers (Chapter 3) suggests that the tobacco cues, like the unpleasant cues, may have energized escape-motivational networks in the anterior cingulate. However, the smokers rated the tobacco cues as appetitive during Visit 3, which raises the possibility that the tobacco cues could have energized appetitive-motivational networks in the anterior cingulate, possibly via input from mesocorticolimbic dopaminergic fibers. Thus, future research is necessary to further explore the discrepancy between self-report and physiological response, which may help to disambiguate the affective nature of the BOLD response to tobacco cues in the anterior cingulate.

Tobacco-specific responses in abstinent smokers

A major goal of this study was to disambiguate tobacco-specific cue reactivity from more generalized emotional responses to highly arousing stimuli. Although BOLD cue reactivity in the dorsal striatum was common to unpleasant and tobacco cues, several brain regions showed tobacco-specific cue reactivity. The [abstinent >

nonabstinent] contrast was significant for [tobacco > neutral], but not [pleasant > neutral] or [unpleasant > neutral], cue reactivity in several areas in the medial prefrontal cortex. These regions included the medial and superior frontal gyri, which have been shown to be active in abstinent smokers in other studies (David et al., 2005; Janes et al., 2009; McClernon et al., 2009). This supports the conclusion that, although these studies only compared tobacco and neutral cues, the responses that were observed in medial prefrontal cortex may have been specific to the tobacco-related stimulus content. This agrees with theories of addiction that predict that drug-related emotional responses are mediated, in part, by orbitofrontal and medial frontal areas (Koob & Volkow, 2010).

We also found evidence of specific [tobacco > neutral] cue reactivity in the temporal lobe, including the right hippocampus, left fusiform gyrus, and bilateral middle temporal gyri. These structures have also been found to show significant BOLD cue reactivity in previous studies (Due et al., 2002; Janes et al., 2009). Several inferior temporal structures are involved in cognitive and emotional processing of complex visual stimuli. For example, the hippocampus mediates the expression of emotional responses to conditioned cues (e.g., Buchel, Dolan, Armony, & Friston, 1999), which is consistent with our finding that cigarette cues – which presumably have become conditioned stimuli for the effects of smoking – produced elevated BOLD responses in the right hippocampus. The fusiform gyrus is thought to be important in the processing of complex visual cues, especially those from classes of objects with which an individual has had extensive experience (e.g., expert-level discrimination between similar objects; Gauthier, Skudlarski, Gore, & Anderson, 2000). Assuming that

smokers are cigarette experts, it is reasonable to conclude that visual depictions of cigarettes would recruit processing resources in the fusiform gyrus.

Potential Limitations and Future Directions

The main conclusions drawn from this study – that the dorsal striatum may be involved in cue reactivity when drug is not immediately available and that several regions of prefrontal and inferior temporal cortex appear to produce tobacco-specific BOLD responses – must be interpreted within the context of its limitations. A clear limitation of this study is that our sample consisted of lighter smokers than most BOLD cue reactivity studies. This raises the question of whether the smokers were truly "dependent" on nicotine (cf. Heatherton et al., 1991) and whether they were experiencing a "true withdrawal" syndrome. However, the abstinent smokers in this study had statistically significant increases in self-reported cigarette craving, anxiety, and desire to smoke to avoid withdrawal, and trend-level significance for increases in other withdrawal symptoms. Furthermore, many of the brain regions that showed significant signal change in our study agree with the findings of cue-reactivity studies of heavy smokers (e.g., David et al., 2005; Due et al., 2002; Franklin et al., 2007; Janes et al., 2009; McClernon et al., 2009). Finally, it should also be noted that even the lightest (Davies et al., 2000; O'Loughlin et al., 2003; Sayette et al., 2001) and most inexperienced (DiFranza et al., 2000; Kassel et al., 2007) smokers experience withdrawal symptoms and have difficulty quitting smoking, and that these groups are vulnerable to transition from light to heavy smoking (Chassin et al., 1996; Wetter et al., 2004), which makes it important to study cue reactivity in these populations.

It may also be difficult to generalize the results of this study to the general population because of the small sample size. Although our sample size was within the bounds for sample sizes in traditional between-groups fMRI studies, recent studies have included more participants (Huettel, Song, & McCarthy, 2004). This raises the concern that some of our findings may be an artifact of the small sample size. Nevertheless, several factors support the validity of our findings. These include our replication of the well-established effect of larger whole-brain volumes of activation for emotionally evocative pictures, compared to neutral pictures (Fig 5-2.; Bradley et al., 2003; Lang, Bradley, Fitzsimmons et al., 1998; Sabatinelli, Lang et al., 2007) and our replication of several tobacco cue reactivity effects seen in previous studies. Our use of a random effects model to test for between-groups differences also suggests that our significant results can be generalized beyond the current sample (Holmes & Friston, 1998).

Our small sample size also precludes individual differences analysis, such as investigating effect of abstinence- and cue-induced changes in craving and anxiety on the BOLD response to emotional pictures. Previous research has found significant correlations between self-reported cocaine craving and dorsal striatal dopamine responses to cocaine cues (Volkow et al., 2006), but not between cigarette craving and BOLD responses to tobacco cues. Thus, future investigation of larger samples is necessary to elucidate the relationship between craving and other subjective reports (such as anxiety and withdrawal symptoms) and neural responses to smoking cues.

A final, potential limitation of this study is that it the voxelwise analysis and necessary cluster-size-thresholding procedure may have prevented the detection of

small areas of activation. Many areas that are thought to be related to drug-related emotional responses, such as the amygdala (e.g., Koob & Volkow, 2010) and habenula (e.g., Taraschenko, Shulan, Maisonneuve, & Glick, 2007), are small and therefore undetectable using a 10-voxel (270 mm^3) threshold. Importantly, the ventral striatum is detectable using comparable cluster sizes to the one used here (McClernon, Kozink, & Rose, 2008), which suggests that our negative finding in that region was reliable. However, future analyses using techniques such as Region of Interest analysis would be advantageous in the study of BOLD cue reactivity.

Conclusion

In this study, we found significant BOLD cue reactivity in the dorsal striatum and anterior cingulate cortex to tobacco and unpleasant pictures in abstinent smokers. This finding may suggest a new role for the dorsal striatum in the neurobiology of addiction: mediating negative affective cue reactivity during periods of abstinence when smoking is not an immediate possibility. This may be relevant to the study of smoking-cessation therapies, as one of the biggest challenges facing smokers is to remain abstinent in the presence of smoking and negative affective cues (Shiffman et al., 1996). Thus, future research into the relationship between dorsal striatal cue reactivity and smoking cessation outcome is recommended.

CHAPTER 6: GENERAL DISCUSSION

The goal of this dissertation was to develop a translational model of tobacco addiction that can be applied to neuroscientific and treatment development studies. This model uses emotion modulated startle as a measure of affect in both rats and humans. In rats, withdrawal-potentiated startle emerged after 1 week of daily nicotine injections, which is suggestive of an escalation in the severity of a negative affective withdrawal syndrome over the course of repeated exposures to nicotine. In humans, smokers who were 24 h into a 48-h abstinence period showed robust fear-potentiated startle to unpleasant cues, while nonabstinent smokers did not show fear-potentiated startle to the same cues. Event-related potentials and peripheral psychophysiology also indicated that the abstinent smokers were more emotionally reactive to unpleasant pictures than nonabstinent smokers. These results suggest that the smokers were in a state of heightened negative affect when deprived of nicotine, which resembles the results of the rat model.

Implications

These studies have several implications. First, the results provide support for negative reinforcement models of tobacco addiction. Second, they suggest that potentiated startle may be a promising translational model of tobacco addiction. Third, the fMRI data provide further support for the role of the anterior cingulate and dorsal striatum in tobacco addiction.

Support for negative reinforcement models

The emergence of withdrawal-potentiated startle in rats and greater fear-potentiated startle in abstinent smokers than nonabstinent smokers are supportive of negative reinforcement theories of tobacco addiction. These theories postulate that the primary determinant of smoking is avoidance of negative affective states, especially anxiety, that arise from abstinence symptoms, stress, or smoking cues (e.g., Baker et al., 2004; Koob & Le Moal, 2001, 2008; Koob & Volkow, 2010; Poulos et al., 1981; Solomon & Corbit, 1973; Watkins, Koob et al., 2000; Wikler, 1973). Our findings provide evidence of increased negative affect in all three situations.

The rodent model suggests that the tobacco abstinence syndrome itself produces an anxiety-like state in the absence of explicit emotional cues. This negative affective state, indexed by elevated startle amplitude peaking 2 h after nicotine injection, developed over the course of repeated nicotine exposures. Escalation of withdrawal-potentiated startle agrees with models that predict that affective withdrawal symptoms emerge as a result of neuroadaptive changes that culminate in increased activity in the extended amygdala during withdrawal (Koob & Le Moal, 2001).

I also found evidence of greater negative affective responses to aversive stimuli in abstinent smokers than in nonabstinent smokers. Smokers who were 24 h into a 48-h deprivation period showed robust fear-potentiated startle to unpleasant pictures, while those who had just smoked a cigarette did not. Similarly, rats that had just been injected with nicotine had lower startle amplitude during the 5-min test session than those that had just been injected with saline. Both of these findings suggest that nicotine has

potent anxiolytic effects, and agrees with self-reports of smokers who smoke to reduce negative affect (Parrott, 1993, 1995; Piasecki et al., 1998; Piasecki et al., 1997; Piasecki et al., 2000). The startle probe P3 and picture-onset ERP findings also suggest that smoking a cigarette reduced, or that withdrawal increased, reactivity to unpleasant stimuli. Collectively, these results support the prediction of negative reinforcement models that smoking is reinforced, in part, because it reduces negative affect (e.g., Baker et al., 2004).

Negative reinforcement models also predict that smoking cues promote relapse by eliciting a negative affective state, such as conditioned withdrawal (Poulos et al., 1981) or frustrative non-reward (Drobes et al., 2001). Contrary to the predictions of these models, we found no difference in startle amplitude to tobacco cues between nonsmokers, nonabstinent smokers, and abstinent smokers. Similarly, other studies have found no evidence of potentiated startle to tobacco cues in abstinent or nonabstinent smokers (e.g., Cinciripini et al., 2006; Geier et al., 2000; Orain-Pelissolo et al., 2004). Using psychophysiological measures other than startle (i.e., startle probe P3 suppression, picture onset ERP), we found that abstinent smokers' responses to tobacco cues closely resembled those to unpleasant pictures, suggesting that these cues may have elicited an aversive motivational state. However, other measures (e.g., zygomatic EMG, self-report) indicated that smoking cues were appetitive to abstinent smokers, which is more consistent with positive reinforcement models of tobacco addiction (Eikelboom & Stewart, 1982; Geier et al., 2000; Stewart, de Wit, & Eikelboom, 1984). Thus, ambiguity remains with respect to the affective nature of

smokers' responses to tobacco cues, and future research is necessary to resolve this important issue.

Potentiated startle as a translational model of tobacco addiction

The results from these studies support the use of potentiated startle paradigms as a translational model of tobacco addiction. Potentiated startle has several advantages in the study of addiction. First, the effects that we observed in rats and humans were large, and became statistically reliable with relatively small sample sizes. This is advantageous for neuroscientific research in animals and in the early phases of clinical trials, in which sample sizes are typically constrained by the complexity or cost of the procedures. Second, the quantification of emotion-modulated startle as a percent change or difference score from the control condition (pre-injection in rats, neutral pictures in humans) allowed the subjects to serve as their own controls (Lang et al., 1990; Walker & Davis, 2002). Third, elicitation of the startle reflex was under the control of the experimenter via timed presentation of startle probes. This means that startle amplitude can be time-locked to the acquisition of neurobiological data (e.g., ERPs), which is difficult using other measures of emotional behavior because the experimenter must wait for the subject to emit a response (Davis, 1986). Fourth, it is possible to measure startle amplitude to probes with different intensity levels (as I did in the rat model), which provides a way of verifying that the basic startle circuit, independent of emotion-modulation, is unaffected by the experimental manipulation (i.e., startle amplitude should be greater for more intense stimuli regardless of the type of emotional stimulus being presented; Blumenthal & Berg, 1986; Davis, 1986). Fifth,

emotion-modulated startle persists as overall startle amplitude habituates, with similar [emotional – neutral] difference scores both early and late in testing (Bradley, Lang, & Cuthbert, 1993). This suggests that startle amplitude may be a superior measure for experiments that require repeated testing over time, as is the case in many clinical trials.

Perhaps the most important advantage of using potentiated startle paradigms is that the neuroanatomy and neuropharmacology of potentiated startle is well-mapped in animals, and is presumed to be similar in humans (e.g., Davis, 2000). This allows the formation of specific, testable hypothesis on the basis of startle results. For example, the CeA is involved in the expression of fear-potentiated startle to explicit cues while the BNST is involved in the expression of fear-potentiated startle to contextual cues (e.g., Walker & Davis, 1997b; Walker et al., 2003). Thus, a reasonable hypothesis that can be easily investigated in rats is that withdrawal-potentiated startle in the absence of cues (as was seen in Chapter 2) will be sensitive to BNST lesions or inactivation, whereas withdrawal-induced changes in fear-potentiated startle (Chapter 3; cf. Fendt & Mucha, 2001) will be sensitive to CeA lesions or inactivation.

Neurobiological findings

In Chapter 1, I outlined four neurobiological systems that are thought to be important in addiction. These were the mesocorticolimbic dopamine system, the extended amygdala, the anterior cingulate gyrus and prefrontal cortex, and the dorsal striatum. The fMRI experiment (Chapter 5) directly implicated the involvement of two of these systems in abstinent smokers' responses to smoking-related and emotional cues: the anterior cingulate gyrus and dorsal striatum. Significant cue reactivity to

tobacco and unpleasant cues in the head of the caudate in abstinent smokers, but not in nonabstinent smokers or nonsmokers, is a potentially important finding. For the most part, the dorsal striatum has been described as being involved in the habitual, but not affective, aspects of drug-taking (Everitt et al., 2008; Everitt & Robbins, 2005). Recently, however, this study and others (McClernon et al., 2009; Volkow et al., 2006) have found evidence to suggest that the caudate may be involved in addicts' emotional responses. This agrees with new theories of dorsal striatal function that suggest that the medial and anterior subdivisions of the caudate may be involved in the acquisition and expression of motivated, response-outcome associations (Balleine et al., 2007). Thus, it is important to conduct additional research into the role of the caudate in the motivational aspects of cigarette smoking.

The findings of withdrawal-potentiated startle in rats, robust fear-potentiated startle to unpleasant cues in abstinent smokers, and no fear-potentiated startle to the same cues in nonabstinent smokers suggests that the extended amygdala is also involved in abstinence-induced negative-affect. Increases in startle probe P3 suppression to tobacco and unpleasant pictures during abstinence and decreases in late positive potentials to tobacco, pleasant, and unpleasant pictures immediately after smoking a cigarette indicate that posterior parietal circuits that are involved in motivated attention may also be important in the expression of smoking-related emotional responses. These results, summarized in Figure 6-1, suggest that the paradigms described in this dissertation will be useful in future investigations of the

involvement of these brain systems in the development and maintenance of cigarette addiction.

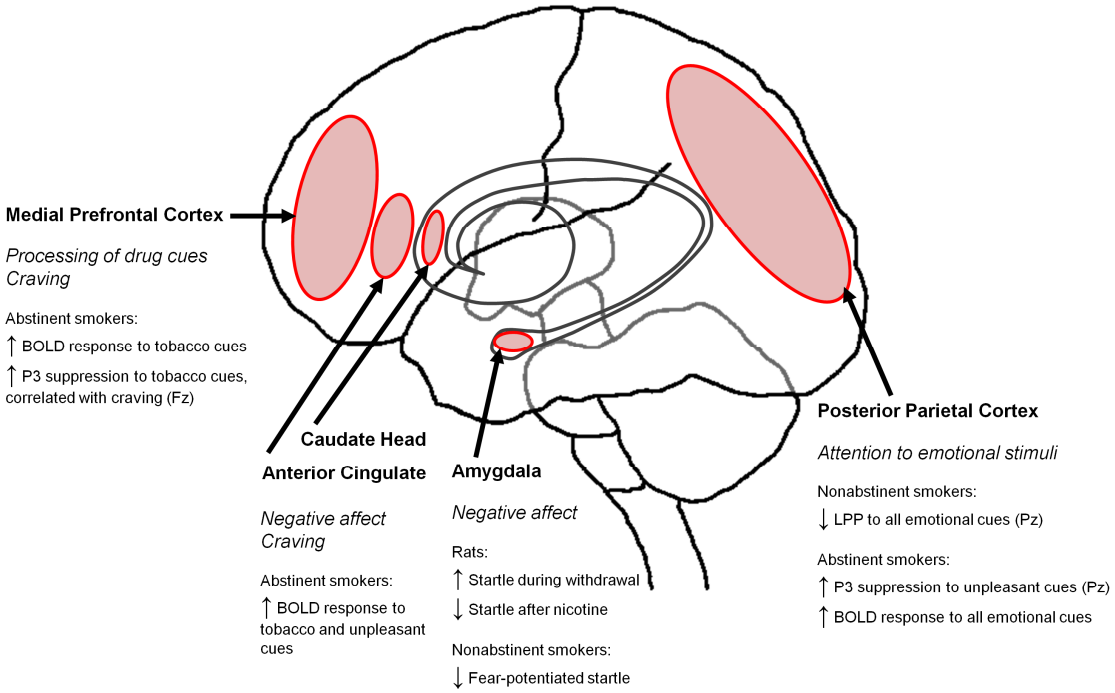

Figure 6-1. Summary of the findings from all experiments in terms of neural systems that are hypothesized to be important in their expression. *Shaded areas* provide a schematic representation of brain regions that may be important in the smoking-related emotional responses observed in this study. The names of these regions are indicated in *boldface* text. *Italicized text* describes the psychological phenomena that may be mediated by these regions, and *plain text* summarizes empirical findings from the rat and human models that support the role of the indicated brain regions in smoking-related emotional responses.

Directions for Future Research

In addition to the directions for future research that were discussed with respect to the individual experiments presented in Chapters 2-5, synthesis of the results from all of the experiments leads to several questions for additional investigation. These include 1) the role of emotional cues in rodent withdrawal-potentiated startle, 2) the need to further study the effect of smoking availability on emotional response, 3) addressing discrepancies between self-report and psychophysiology, 4) extending the model to a

broad population of smokers, 5) assessing the effects of repeated, within-subjects testing, 6) using the model to study new stop-smoking therapies, and 7) using results from human functional brain imaging studies to guide animal research.

Examine the role of emotional cues in rodent withdrawal-potentiated startle

In the rat model, withdrawal-potentiated startle was evident in the absence of explicit emotional cues. In the human model, there were no significant between-group differences in startle in the absence of cues; withdrawal-induced changes in startle (and other psychophysiological responses) were only evident in response to discrete emotional cues. This is a critical difference between the models, and it may pose significant challenges for translational research.

In humans, it is difficult to reliably detect changes in basal startle amplitude in the absence of explicit cues. Thus, the easiest solution to the issue of the cues used in the model is likely to be the inclusion of explicit emotional cues in the rodent model. This can be accomplished by testing the effects of nicotine administration and withdrawal on the expression of fear-potentiated startle. For example, a Pavlovian fear conditioning phase (e.g., light-shock pairings) can be added to the rat model prior to the nicotine exposure phase. Then, fear-potentiated startle can be measured at various time points after the nicotine injection to determine whether withdrawal modulates fear-potentiated startle, similar to the effects of tobacco abstinence on fear-potentiated startle to unpleasant pictures that were observed in the smokers.

Interestingly, fear-potentiated startle to explicit cues increases during opiate withdrawal (Fendt & Mucha, 2001). Given the similarity of the affective signs of

nicotine and opiate withdrawal (Hughes et al., 1994), nicotine withdrawal is also likely to increase fear-potentiated startle, an effect that would be consistent with the smokers' responses to unpleasant pictures in the human model. It has also been shown that rats withdrawing from chronic, continuous nicotine had increased light-enhanced startle (Jonkman et al., 2008), a measure of unconditioned fear in which the startle reflex is measured in response to long-duration, bright lights (Walker & Davis, 1997a). This finding suggests that, in rats, nicotine withdrawal increases startle responses to emotional cues in addition to the potentiation of basal startle amplitude reported in Chapter 2 and by others (e.g., Helton et al., 1993). Furthermore, withdrawal-induced increases in anxiety-like responses in paradigms such as defensive burying, the elevated-plus maze, conditioned place aversion, and the open-field test also suggest that fear-potentiated startle will increase during nicotine withdrawal (Bhattacharya et al., 1995; George et al., 2007; Irvine et al., 2001; Pandey et al., 2001; Suzuki et al., 1996; Tzavara et al., 2002).

The second option to resolving the difference between the rat and human model would be to develop a human paradigm that more closely resembles the rat paradigm, i.e., to monitor startle amplitude in the first few hours after smoking a cigarette, as opposed to 24 h later. It is possible that changes in startle amplitude in the absence of emotional cues are more likely to be observed at the beginning of a withdrawal episode, when brain nicotine levels are falling. As withdrawal progresses and nicotine levels are minimal, an emotional challenge, such as presentation of cues or stressors, may be required to produce changes in startle amplitude (Jonkman et al., 2008).

Pharmacological challenges may also produce withdrawal-potentiated startle at later time points. We have found that naloxone, an opioid receptor antagonist, potentiates startle after nicotine exposure in rats (Engelmann, Radke, & Gewirtz, unpublished data). Thus, a similar pharmacological challenge in humans may provide an additional methodology for measuring the effect of nicotine withdrawal on startle amplitude.

Finally, it should be noted that there is no inconsistency between the results of the rat and human models. Rather, somewhat different startle paradigms (i.e., early in withdrawal without explicit cues vs. late in withdrawal with explicit cues) were used in order to make the studies consistent with previous studies in each field (e.g., Cinciripini et al., 2006; Drobes et al., 2001; A. C. Harris & Gewirtz, 2004; Helton et al., 1993). The results of the rat and human paradigm are entirely compatible, and the studies proposed here (i.e., measuring startle responses to explicit cues in rats and earlier in withdrawal or in response to pharmacological challenge in humans) have the potential to demonstrate exactly how the original rodent and human paradigms presented in Chapters 2 and 3 are related.

Directly assess post-session smoking availability

The factor of post-session smoking availability was used to explain differences between the current model, which tested smokers 24 h into a 48-h abstinence period, and existing models, which tested smokers at the end of a short deprivation period. The hypothesis that the dorsal striatum and ventral striatum may have different functions that are dependent on post-session smoking availability is especially intriguing. However, the current set of experiments did not include a direct assessment of the

effects of smoking availability on abstinent smokers' psychophysiological and BOLD responses. Thus, an important step for future research is to directly compare psychophysiological and BOLD cue reactivity between two groups of smokers: those who are 24 h into a 48-h deprivation period and those who are at the end of a 24-h deprivation period and told that they can smoke at the end of the session.

Address discrepancies between self-report and psychophysiology

In both the psychophysiology and fMRI study, nonabstinent and abstinent smokers rated the tobacco pictures as pleasant. Psychophysiological and fMRI data revealed responses that were more similar to unpleasant pictures than pleasant pictures, and thus the tobacco pictures were interpreted as aversive. This discrepancy between self-report and psychophysiology is not surprising, as self-report and physiological response often diverge (e.g., Lang et al., 1990; Lang, Bradley, & Cuthbert, 1998). Furthermore, self-report ratings were obtained during Visit 3, at the end of the abstinence period, whereas physiological responses were measured during Visit 2, in the middle of the abstinence period. It is possible to obtain real-time valence and arousal ratings simultaneously with psychophysiological recording, and this type of experiment would help to address whether the differences in ratings of tobacco pictures was the result of obtaining the ratings at the end of the experiment. However, obtaining ratings at the same time as psychophysiological recording has been shown to influence physiological responses (B. N. Cuthbert lab, unpublished data), possibly due to increased attention to the stimuli that accompany the ratings task. Thus, results from

future studies in which ratings are obtained closer in time to psychophysiological recording must be interpreted with care.

Apply this model to a broad population of smokers

The current studies used light to moderate smokers, whereas existing cue reactivity studies have used heavy smokers, who smoke at least 20 cigarettes per day, on average. Our use of lighter smokers provides a unique contribution to the literature, and provides further support for the hypothesis that negative affective abstinence symptoms emerge relatively early in the course of tobacco addiction and are a critical factor in the transition to compulsive drug use (Davies et al., 2000; Kassel et al., 2007; Koob & Le Moal, 2001; O'Loughlin et al., 2003; Sayette et al., 2001; Solomon & Corbit, 1973). However, most smokers who seek stop-smoking therapy are heavy smokers who have been smoking for decades. It is therefore important to recruit smokers from this population in future tests of the cue reactivity model. By mapping psychophysiological and BOLD responses to emotional stimuli across the course of the addiction process, it will be possible to develop a clearer picture of the neuroadaptive changes that are thought to be important in the development of tobacco addiction, which may help in the identification of effective smoking-prevention interventions (e.g., nicotine vaccine; Cerny & Cerny, 2009) in addition to stop-smoking therapies.

Examine the effects of repeating the model within-subjects

In most clinical trials, the same measurements are acquired at multiple time points (e.g., pre- and post-treatment). Thus, if the cue reactivity paradigm developed in this study is to be used in clinical trials, it needs to be measured at multiple time points.

Future research should therefore determine the stability of the psychophysiological and BOLD responses to the tobacco, pleasant, neutral, and unpleasant pictures across multiple sessions, some of which are months or years apart. As mentioned above, overall startle amplitude decreases across multiple test sessions (Ornitz & Guthrie, 1989). Emotion-modulated startle, when quantified as a difference score or percent change from neutral pictures typically remains stable, however, which suggests that the emotion-modulated startle findings will be consistent across sessions (Bradley et al., 1993).

An additional concern that arises with repeated testing is that participants may find the pictures less arousing with multiple viewings. It appears, however, that repeated presentation of pictures, especially when delivered in a random order with respect to content category, has little effect on the magnitude of ERPs, SCRs, and heart rate responses to picture onset (Bradley, 2009). The stability of the BOLD signal to repeated presentation of emotional pictures has not been systematically examined, but BOLD responses to simple visual stimuli appear to be consistent across multiple scanning sessions (Noll et al., 1997). Also, the stability of ERPs to repeated presentation of emotional pictures suggests that the BOLD response will remain stable over time, because both ERPs and BOLD responses appear to be the result of the same underlying neural activity (Sabatinelli, Lang et al., 2007).

Use this model in clinical investigations of smoking-cessation treatments

The ultimate goal of developing the rat and human withdrawal-potentiated startle paradigms is to provide a tool for the assessment of new stop-smoking therapies.

The next step in the process of translating this laboratory model to clinical settings is to assess the effects of existing stop-smoking medications (e.g., bupropion, varenicline) on withdrawal-potentiated startle and other psychophysiological responses to determine whether a certain pattern of physiological response predicts treatment outcome. After establishing predictive validity of a psychophysiological measure by applying it to existing medications, the same measure can be used in the assessment of new medications.

In addition to being used as a predictor of medication efficacy, psychophysiological and BOLD responses can be used to identify potential mechanisms of action of effective smoking cessation medications. For example, consider a medication that is effective at facilitating successful quit attempts that also reduces fear-potentiated startle to aversive conditioned stimuli in rats and to unpleasant pictures in abstinent smokers. This type of result would suggest a mechanism of action that involves the reduction of withdrawal-induced anxiety, presumably via the extended amygdala. The search for improved medications could then focus on their ability to produce greater reductions in fear-potentiated startle, possibly by targeting nAChR subtypes that are localized to the amygdala (Dwoskin et al., 2009; Leonard & Bertrand, 2001). A simple but powerful laboratory model, such as the paradigms described in this dissertation, is the first step in realizing this promising approach to medication development (Lerman et al., 2007).

Use the human model to guide animal research

Finally, it is important to consider "reverse translational research", in which findings from the human model guide further animal research. The results from the emotional cue reactivity study provide several new directions for animal research. First, the finding of significant fear-potentiated startle to unpleasant pictures in abstinent smokers and no fear-potentiated startle to unpleasant pictures in nonabstinent smokers suggests that the extended amygdala may be involved in withdrawal-induced increases and nicotine-induced decreases in negative affect. Thus, the amygdala can be directly manipulated in rats that are deprived or not deprived of nicotine. The effects of these amygdala manipulations on withdrawal-potentiated startle and fear-potentiated startle might provide insight into the specific structures or neurotransmitter systems within the extended amygdala that are involved in the expression of withdrawal-induced negative affect (cf. A. C. Harris et al., 2006).

Second, the brain areas that showed significant cue reactivity effects in the fMRI study may provide additional targets for direct manipulation in rodents. Because most of these areas were significantly active in abstinent smokers who were viewing tobacco or unpleasant pictures, incorporation of aversive or nicotine-related cues in the rat experiments is encouraged. For unpleasant cues, this can be accomplished via measurement of fear-potentiated startle. For tobacco cues, options include measuring the effects of neural manipulations on cue-induced reinstatement of nicotine self-administration, conditioned place preference, or conditioned place aversion. An especially promising option may be to measure startle amplitude in the presence of

stimuli that have been used to signal nicotine availability during self-administration sessions. Then, the effects of neural manipulations on startle amplitude to these discriminative stimuli can be measured in regions of interest that showed [tobacco > neutral] cue reactivity in the human fMRI study. Such studies would further illustrate the bidirectional translation that is possible when using emotion-modulated startle paradigms, and demonstrate the power of the models developed in our laboratory.

Conclusion

The research described in this dissertation included the development of a rodent model of the anxiety-like aspects of nicotine withdrawal using the potentiated startle paradigm, and the translation of that model to emotion-modulated startle in human smokers. The human laboratory model went beyond the startle reflex methodology to include event-related potential, peripheral psychophysiological, and fMRI approaches.

For years, researchers have stressed the importance of interdisciplinary, translational models of tobacco addiction in the search for more effective smoking prevention strategies and smoking cessation treatments. The model described here provides a cross-disciplinary and cross-species model of the affective aspects of tobacco addiction, and provides a first step in the development of the long-sought translational model. Expansion upon these findings may lead to exciting advances in the neuroscience of addiction and in the identification smoking-cessation interventions.

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