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EVALUATING THE ACUTE EFFECTS OF CAFFEINATED WATERPIPE TOBACCO IN
WATERPIPE USERS

A dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy at Virginia Commonwealth University.

by

CAROLINE O. COBB
M.S., Virginia Commonwealth University, 2009

Director: Dr. Thomas Eissenberg
Professor
Department of Psychology and Institute for Drug and Alcohol Studies

Virginia Commonwealth University
Richmond, Virginia
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Acknowledgements

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List of Abbreviations

ANOVA	analysis of variance
CDC	Centers for Disease Control and Prevention
CO	carbon monoxide
HSD	Honestly Significant Difference
LOQ	limit of quantification
mg	milligram
min	minute
ml	milliliter
ng	nanogram (0.0000000001 grams)
ppm	concentration in parts per million
US DHHS	U.S. Department of Health and Human Services
VAS	visual analog scale
VTA	ventral tegmental area
μg	microgram

Abstract

EVALUATING THE ACUTE EFFECTS OF CAFFEINATED WATERPIPE TOBACCO IN
WATERPIPE USERS

By Caroline O. Cobb, M.S.

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2012.

Major Director: Dr. Thomas Eissenberg
Professor
Department of Psychology and Institute for Drug and Alcohol Studies

Caffeine and nicotine are the two most commonly consumed licit psychoactive drugs in

the world. In addition, they are frequently co-administered with over 86% of cigarette smokers

reporting caffeine use versus 77% of non-smokers. Research suggests the combination of

nicotine and caffeine produces effects that are more rewarding or pleasurable than either drug

alone, and this potential reward enhancement may influence patterns of tobacco use initiation and maintenance. Waterpipe tobacco smoking is an alternative tobacco use method that is increasing in prevalence in the U.S. and offers a novel opportunity for nicotine and caffeine co-administration via a caffeinated tobacco product (Tangiers F-Line). Based on previous work, this caffeinated tobacco product was hypothesized to enhance reward-related and cardiovascular effects in waterpipe users relative to tobacco-only waterpipe preparations. Thirty-two waterpipe tobacco smokers who regularly drank caffeinated beverages participated in a four condition, Latin-square ordered, within-subjects study. In each condition, there was a 45-minute double-blind product administration period that differed by the content of waterpipe product smoked: caffeine and nicotine (Tangiers F-Line), nicotine and no caffeine (Tangiers), reduced (low) nicotine and caffeine (low nicotine Tangiers F-Line), or neither nicotine nor caffeine (Soex). Outcome measures included blood plasma caffeine and nicotine, cardiovascular response, expired air carbon monoxide (CO), puff topography, and subjective ratings. Plasma analyses revealed no detectable levels of caffeine from either caffeinated product, but significant nicotine exposure from all nicotine-containing products. Few differences between conditions were observed for subjective measures. Larger puff volumes were observed for products that contained low or no nicotine, resulting in higher CO concentrations for these conditions. While findings do not address whether caffeine can be delivered via volatilization, they suggest that measurable caffeine exposure was not observed for the products examined and under the conditions explored here. Importantly, study results support continued investigation of the effects of waterpipe tobacco smoking using a placebo-controlled design as well as demonstrate that

tobacco dependence and toxicity capabilities are still concerns for these and other waterpipe

products.

Evaluating the acute effects of caffeinated waterpipe tobacco in waterpipe users

Overview

Caffeine and nicotine are the two most commonly consumed licit psychoactive drugs in the world (Frary, Johnson, & Wang, 2005; Tanda & Goldberg, 2000). Both are considered stimulants and share some similar physiological and behavioral effects. Nicotine is usually administered via tobacco products and caffeine is most often consumed orally via beverages. While tobacco use is associated with nicotine dependence and serious health effects, caffeine use is considered a behavior with low abuse liability and little harm (United States Department of Health and Human Services [USDHHS], 2004; Daly & Fredholm, 1998). The co-administration of caffeine and nicotine-containing products is common: over 86% of cigarette smokers report using caffeine versus 77% of non-smokers (Swanson, Lee, & Hopp, 1994). This observation may reflect an interaction between these two drugs such that their combination produces more rewarding or pleasurable effects than either drug alone. This reward enhancement may influence patterns of tobacco use initiation and maintenance (Tanda & Goldberg, 2000; Jones & Griffiths, 2003; Gasior, Jaszyna, Munzar, Witkin, & Goldberg, 2002), posing a significant risk to potential and current tobacco users. Although cigarette smoking prevalence has been decreasing over the past twenty years in the United States (U.S.), the recent popularity of an alternative tobacco use method, waterpipe tobacco smoking, also raises concerns over nicotine and caffeine co-administration (Centers for Disease Control and Prevention [CDC], 2008; World Health Organization, 2005). This method of tobacco smoking, especially popular among young adults

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in the U.S., may allow for increased opportunities for nicotine and caffeine co-administration among an age group that is especially vulnerable to tobacco use (CDC, 1994). One such opportunity presents itself with the availability of a caffeinated waterpipe tobacco product, that enables users to consume this combination of drugs in a single form. While the individual effects of nicotine and caffeine are well-defined, empirical research concerning their interaction, reward enhancement, and influence on patterns of tobacco use initiation and maintenance is less clear. Furthermore, no data exist concerning the effects of caffeine delivered via tobacco. By examining the individual and combined effects of nicotine and caffeine, as well as developing a technique to assess caffeinated tobacco products, their influence on patterns of tobacco use initiation and maintenance may be better illustrated.

Nicotine's Pharmacokinetics and Pharmacodynamics

Nicotine is a mild psychomotor stimulant found in tobacco and produces subjective effects similar to other drugs of this classification including cocaine (Jones, Garrett, & Griffiths, 1999), amphetamine (Grilly, 2000), and caffeine (Garrett & Griffiths, 2001). While nicotine is the primary chemical responsible for tobacco dependence, other tobacco or tobacco smoke constituents such as the tobacco specific nitrosamines and polycyclic aromatic hydrocarbons (Hecht & Hoffman, 1998; Hecht, 2006) are implicated in the development of damaging health effects that include various cancers, pulmonary disease, and adverse pregnancy-related outcomes (USDHSS, 2004). Understanding how the pharmacological properties associated with nicotine influence the use of tobacco despite these harmful consequences is vital to decreasing the impact this drug has upon public health.

Specifically, nicotine's pharmacokinetics and pharmacodynamics are important factors in the effects this drug has upon patterns of tobacco use initiation and maintenance. Nicotine is absorbed easily from multiple sites in the body including the lungs, mucosa, skin, and gastrointestinal tract (Julien, 2005, p. 233; Benowitz, 2008). Tobacco smoke contains nicotine in the form of minute particles that are quickly absorbed into the bloodstream from alveoli in the lungs (Zevin, Gourlay, & Benowitz, 1998). During smoking, nicotine reaches the brain within about 10 to 20 seconds (Benowitz, 2008), while peak plasma levels of nicotine are observed about 5-10 minutes after inhalation (Balfour & Fagerström, 1996). Brain and plasma levels of nicotine decline quickly due to elimination and distribution to peripheral tissues. Contributing to the speed of elimination is nicotine's short half-life, which is approximately two hours in most adults (Benowitz, Kuyt, & Jacob, 1982; Benowitz, 2008). Cotinine, the primary metabolite of nicotine, has a much longer half-life (14-20 hours) and is often used as a biomarker of smoking status (Zevin et al., 1998; Benowitz & Jacob, 1993). This rapid absorption and elimination profile has important implications for nicotine's ability to serve as a drug of abuse for many individuals. In order to maintain the drug's pleasurable effects and prevent symptoms associated with tobacco abstinence, continued nicotine self-administration (e.g., smoking) is necessary (USDHHS, 2010).

Nicotine affects a variety of structures in the central nervous system (CNS), peripheral nervous system (PNS), and the heart. Physiological effects include increases in heart rate, blood pressure, and cardiac contractility (Benowitz, Porchet, Sheiner, & Jacob, 1988; Zevin et al., 1998). Behavioral effects include increases in fine psychomotor activity (Perkins et al. 1990),

attention (Bates, Mangan, Stough, & Corballis, 1995), and cognitive task performance (Foulds et al., 1996). Nicotine exerts a majority of its action by activating specific acetylcholine receptors, which are distributed throughout the CNS and PNS (nicotinic acetylcholine receptors; Golan et al., 2005, p. 257; Benowitz, 2008). In the CNS, nicotine administration is associated with nicotinic acetylcholine receptor activation in the pre-synaptic nerve terminals of dopamine, acetylcholine, and glutamate secreting neurons (among many others), which when activated by nicotine facilitate the release of these neurotransmitters (Golan et al., 2005, p. 257; Benowitz, 2008). Critical to the reinforcing effects of nicotine and other drugs of abuse is the release of dopamine (Benowitz, 2008).

Nicotine increases dopamine levels in the mesocorticolimbic system of the brain involving the ventral tegmental area (VTA), nucleus accumbens, and forebrain, as well as the corpus striatum and the prefrontal cortex (Corrigal, Coen, & Adamson, 1994; Domino & Tsukada, 2009). These increases may account for some of the acute and chronic effects of nicotine which include stimulant, behavioral reinforcement, and dependence-inducing properties (Picciotto, 1998; Le Foll & Goldberg, 2009; Zevin et al., 1998).

Acute Effects of Nicotine in Humans

Acute nicotine administration in a clinical setting is associated with a reliable set of outcomes that include stimulant-related physiological and subjective effects. Examinations that assess these acute outcomes are usually performed in a controlled laboratory setting using classic measures of physiological activity (i.e., heart rate, blood pressure) and well-defined measures of subjective effects. Within these examinations, nicotine can be administered in a variety of forms

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(e.g., intravenous [i.v.], nicotine gum, nicotine-containing cigarette) and among participants with and without a nicotine use history. The breadth of research concerning the acute effects of nicotine provides ample and consistent evidence of this drug's immediate effects, and understanding these acute effects may inform the study of patterns of tobacco use initiation and maintenance.

Often the acute effects of nicotine in humans are assessed in a single session dosing procedure. One recent study examined the acute cardiovascular and subjective effects of i.v. administered nicotine (Sofuoglu & Mooney, 2009). Twenty-four male and female smokers who were overnight-abstinent from nicotine received a saline infusion followed by 0.5 mg/70 kg and 1.0 mg /70 kg nicotine i.v. The infusions were delivered over 60 seconds and were separated by a 30-minute interval. Physiological measures included blood pressure and heart rate. Subjective effects were assessed using multiple measures including visual scale analog (VAS; 0-100) items (e.g., "drug strength", "head rush", "good drug effects", "like drug" and "bad effects"; Soria et al., 1996) and the 20-item Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988), which contains two factors defined by factor analysis (positive affect and negative affect). Results for heart rate indicated that both active nicotine doses generated significantly greater increases in beats per minute (bpm) as compared with placebo. For diastolic blood pressure, the 0.5 mg/70 kg dose significantly increased blood pressure as compared with placebo, while for systolic blood pressure only the 1 mg/70 kg dose of nicotine produced significantly greater increases. For the subjective measures, significant main effects for dose were observed on all VAS items including "good drug effects", "like drug", and "head rush".

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Scores for all of these items were increased by nicotine administration in a dose dependent manner (i.e., increases were larger after the higher nicotine dose). Women and men reported similar changes in positive affect on the PANAS from the beginning of the session to the end (Sofuoglu & Mooney, 2009). These findings clarify some common effects associated with acute nicotine administration including increased heart rate and blood pressure, and subjective reports of "good drug effects" and "head rush". Importantly, some of these effects are observed in those naïve to nicotine as well.

Non-smokers report some similar effects in response to acute doses of nicotine. In a within-subject laboratory study, the effects of intranasal nicotine doses of 0, 10, and 20 micrograms (μg)/kg were compared between a group of 37 non-smokers and 55 smokers (Perkins, Gerlach, Broge, Grobe, & Wilson, 2000). Doses were administered via measured-dose nasal spray once every 30 minutes for 90 minutes (total of three times per session). Outcome measures included cardiovascular and subjective assessments (e.g., VAS items of "head rush", "relaxed", "pleasant", and "jittery"). Subjective and cardiovascular responses to nicotine doses or placebo were averaged across the three administrations during each session to produce a mean response for each dose tested. Results indicated in both smokers and non-smokers nicotine administration produced dose dependent increases in VAS scales of "jittery" and "head rush", heart rate, and blood pressure (Perkins et al., 2000). These results demonstrate the similarity of some of the acute subjective and cardiovascular effects of nicotine between smokers and non-smokers.

In addition, a meta-analysis examining the acute subjective effects of nicotine in humans including smokers and non-smokers revealed commonalities of acute effects observed across drug use history (Kalman & Smith, 2005). Six nicotine nasal spray studies among non-smokers and eleven nasal spray and four i.v. nicotine studies among nicotine-deprived smokers were included in the analyses. Outcome variables were subjective effects including affective valence (pleasant and unpleasant) and arousal (high and low). Generally, results indicated that in smokers and non-smokers, nicotine produced a decrease in feelings of relaxation and increased tension/jitteriness. Similar to previously reported examinations (Perkins et al., 2000; Sofuoglu & Mooney, 2009), dose-response relationships were observed for some items. The largest effect sizes were for the subjective items "head rush" and "drug high", but there was considerable variability among the studies due to the nicotine doses used and the route of administration (Kalman & Smith, 2005). Overall, these findings suggested that the subjective effects associated with acute nicotine administration are well-defined and can be reliably measured.

In summary, acute nicotine administration produces quantifiable increases in cardiovascular measures including heart rate and blood pressure, as well as some consistent subjective effects. These effects are important as they may be associated with initiation of tobacco smoking and the progression to chronic use. Chronically, nicotine administration may be associated with differing symptoms that influence patterns of tobacco use and maintenance.

Chronic Effects of Nicotine in Humans

Clinical examinations concerning the chronic effects of nicotine are numerous and, similar to acute studies, administration can be achieved via a variety of means (e.g., i.v., nicotine

nasal spray, transdermal nicotine patch). While chronic administration of nicotine is associated with comparable cardiovascular and subjective effects to that of nicotine administered acutely, there are other phenomena associated with chronic nicotine administration. These phenomena include tolerance to the cardiovascular effects of nicotine and abstinence-related effects. Tolerance is an adaptive change of an organism in response to repeated drug administration such that there is a reduced effect and increasingly larger doses are required to produce the same effect obtained with smaller doses (Kalant, LeBlanc, & Gibbins, 1971). Abstinence-related effects are a set of adverse symptoms that can appear when nicotine administration is terminated or reduced, and these effects can be suppressed by re-administration of nicotine (Hughes & Hatsukami, 1986; Breland, Evans, Buchhalter, & Eissenberg, 2002).

Both acute and chronic tolerance to the cardiovascular-related effects of nicotine can be observed in smokers. Acute tolerance is associated with smaller responses to repeated administrations of a drug dose during a single session or short period, while chronic tolerance is indicated by both a history of drug exposure and the observation of a reduced response to a drug dose (Kalant et al., 1971). One study that demonstrates the distinction between acute and chronic tolerance to the cardiovascular effects of nicotine was performed among ten "heavy" ($\geq$ 20 cigarettes per day) smokers and eight "light" ($<$ 20 cigarettes per day) smokers using a two-session mixed study design (Perkins, Epstein, Stiller, Marks, & Jacob, 1989). During sessions, participants received four administrations of nicotine via measured nasal spray every twenty minutes and sessions differed by nicotine dose administered: high nicotine (15 µg/kg approximately equal to a typical cigarette) or low nicotine (7.5 µg/kg). Heart rate was monitored

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during the five minutes following each administration. Analyses of the data collected two minutes after nicotine administration suggested acute tolerance to the nicotine-related increase in heart rate in both groups, which was indicated by a decline in mean heart rate increase from administration 1 to administration 4. For example, the low dose of 7.5 μg/kg at administration 1 produced a mean 6.4 bpm increase and at administration 4 produced a mean 3.9 bpm increase. In modest support of chronic tolerance, mean baseline heart rate did not differ between the light and heavy smokers, but results indicated that relative to the light smokers, heavy smokers had significantly smaller heart rate increases in response to the high doses of nicotine (Perkins et al., 1989). This study indicates that both light and heavy smokers demonstrate acute cardiovascular tolerance, and more frequent self-administration of nicotine is associated with an enhanced tolerance effect.

In addition, tolerance to the subjective effects of chronically administered nicotine has been assessed in the clinical laboratory. A group of eight smokers and seven non-smokers participated in a study consisting of three sessions that differed by nicotine dose administered (0, 7.5, or 15 μg/kg via nasal spray; Perkins et al., 1993). Nicotine doses were given every thirty minutes for two hours, and thirty minutes after the last dose a challenge dose of 30 μg/kg was administered among the smokers only. Prior to each session, participants abstained overnight from nicotine, caffeine, and food. Subjective outcome measures included VAS items assessing "jittery", "light-headed", "relaxed", "dizzy", and "head rush" and specific scales from a mood-related measure, the Profile of Mood States (POMS; McNair, Lorr, & Droppleman, 1971). Results indicated dose-dependent changes in most subjective measures. These changes included

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those observed during acute nicotine studies (e.g., increases in "head rush", "light-headed", and "jittery"). In addition, responses to some of scales/items tended to be smaller in the smokers relative to the non-smokers. For example, among smokers and non-smokers nicotine significantly increased scores on the VAS scales of "lightheaded" and "dizzy", but smokers' responses were significantly smaller relative to those of nonsmokers for these two items. For the item "light-headed" relative to baseline, 7.5 μg/kg of nicotine increased non-smokers' scores approximately 20 points and increased smokers' scores approximately 8 points. In addition, a contrast in subjective effects between smokers and non-smokers was observed for the POMS scales of vigor, tension, arousal, and fatigue. Relative to baseline, nicotine tended to increase smokers' low baseline levels of vigor, decrease their ratings of tension, and had little effects on arousal and fatigue, while nicotine decreased nonsmokers' levels of arousal and vigor and increased fatigue and tension. For example, on the vigor scale, 7.5 μg/kg of nicotine decreased non-smokers' scores approximately 6 points and increased smokers' scores approximately 2 points. These differences may reflect the suppression of abstinence-related symptoms in the smokers, and the induction of adverse nicotine-related symptomology in the non-smokers. This study provides some evidence for the consistency of subjective response to nicotine as well as demonstration of tolerance to the some of the subjective effects of nicotine. Importantly, this study also highlights the effects abstinence-related symptoms and their suppression may incur upon smokers' behavior (i.e., decreased tension and increased vigor; Perkins et al., 1993).

Evidence from the clinical laboratory indicates that aversive tobacco abstinence-related symptoms occur reliably and that administration of a cigarette or pharmacologically pure

nicotine can suppress these symptoms. For example, in an early clinical study of fifty smokers, the signs and symptoms of tobacco abstinence during two days of ad-libitum smoking and during the first four days of tobacco abstinence were examined (Hughes & Hatsukami, 1986). Relative to days during ad-libitum smoking, during abstinence participants reported increases in ratings of anxiety, craving, difficulty concentrating, eating, hunger, impatience, irritability, and restlessness. Decreases in ratings of sleep adequacy and heart rate were also noted during the abstinence phase. To verify these self-reported symptoms, observers were recruited to rate each participant on an identical scale for the following symptoms: anxiety, drowsiness, fatigue, impatience, irritability, restlessness, and somatic complaints. All observer ratings were significantly related to the corresponding participant ratings. This study demonstrated that tobacco abstinence effects can be measured reliably by both participant-reported symptoms and observer-rated signs (Hughes & Hatsukami, 1986). In another clinical examination of tobacco abstinence, participants could press a button on a keyboard to obtain puffs of a cigarette under a progressive ratio schedule (Willner, Hardman, & Eaton, 1995). Participants were either tobacco abstinent or smoked normally for the four hours prior to the session. Significantly increased breakpoints were observed in the tobacco abstinent participants (i.e., abstinent participants were willing to perform more work to obtain cigarette puffs; Willner et al., 1995). These results demonstrate tobacco abstinence symptomology and how tobacco abstinence can influence subsequent nicotine self-administration.

Smoking-induced abstinence symptom suppression has also been studied in the laboratory, and there is little doubt that cigarette smoking and administration of

pharmacologically pure nicotine suppresses abstinence effects. For example, in a short-term clinical examination of 20 cigarette smokers (> 15 per day for the past year) who were required to be overnight tobacco abstinent prior to each session, own brand cigarette administration was associated with a significant decrease on multiple measures of tobacco abstinence including "urges to smoke", "restlessness", "irritability", and "craving a cigarette" (Breland et al., 2002). Similar abstinence suppression effects have been noted in other studies of tobacco abstinent smokers and the changes that occurred during own brand cigarette administration (Buchhalter & Eissenberg, 2000; Breland, Kleykamp, & Eissenberg, 2006). A clinical examination of the effects of transdermal nicotine in smokers abstaining from tobacco/nicotine for at least 8 hours showed that transdermal nicotine induced partial abstinence symptom suppression (Kleykamp, Jennings, Sams, Weaver, & Eissenberg, 2008). A multi-center, randomized, placebo-controlled study conducted among a large group of smokers who quit by using either active or inactive nicotine gum for three days showed that smokers using active nicotine gum experienced significantly greater craving reductions following exposure to smoking cues relative to inactive gum users (Shiffman et al., 2003). These reports support the notion that own brand cigarettes and pharmacologically pure nicotine can suppress tobacco abstinence-related symptomology.

In summary, chronic nicotine administration is associated with an array of effects that are similar to those observed with acute nicotine administration (cardiovascular and subjective) as well as those that are not (chronic tolerance and abstinence-related effects). The relationship between the acute and chronic effects of nicotine and patterns of tobacco use initiation and maintenance may be better illustrated by examining them collectively.

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How Do Nicotine's Acute and Chronic Effects Influence Patterns of Tobacco Use Initiation and Maintenance?

The acute and chronic effects of nicotine have an important bearing on patterns of tobacco use initiation and maintenance. Focusing specifically on cigarette smoking, a primary mode of nicotine self-administration, this behavior can provide pleasurable effects in the short-term while long-term cigarette smoking can lead to adverse effects during abstinence periods. These effects, pleasurable and adverse, encourage both tobacco use initiation and maintenance of tobacco use via positive and negative reinforcement.

Positive reinforcement: cigarette smoking. Because nicotine is a stimulant drug that cigarette smokers self-administer, and because stimulant drugs often act as positive reinforcers (e.g., amphetamine, Rush, Essman, Simpson, & Baker, 2001; caffeine, Griffiths & Woodson, 1988; cocaine, Higgins, Bickel, & Hughes, 1994) initial tobacco use episodes may be reinforced positively (i.e., produce direct effects that make subsequent drug self-administration more likely; Koob & Le Moal, 1997; Koob, 1999). This positive reinforcement may be a result of the acute effects described in the previous section at least some of which are likely mediated by the CNS dopamine system activation (Benowitz, 2008). While preclinical research has demonstrated repeatedly that nicotine administration is associated with dopamine system activation (e.g., Corrigall et al., 1994), recent advances in neuroimaging have enabled researchers to observe real-time changes in human neurobiology in response to nicotine administration via cigarettes. A group of sixty-two smokers participated in a within-subject double blind study to examine dopamine release before and after smoking either a nicotinized or denicotinized cigarette (Brody

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et al., 2009). Positron emission tomography was used to examine neurochemical changes that

occurred in the immediate thirty minutes after smoking. Results indicated that the nicotinized

cigarette produced greater dopamine release relative to the denicotinized cigarette, and increased

dopamine release was associated with more mood improvement (assessed via subjective

measures before and after smoking; Brody et al., 2009). Consistent with other evidence, these

results strengthen the claim that some of the reinforcing properties of nicotine administration

may be mediated by dopamine release.

Other evidence that supports the role of dopamine release in tobacco dependence is the

pharmacotherapy, varenicline. This drug is a selective nicotinic receptor partial agonist, which

causes a moderate and sustained release of mesolimbic dopamine (Coe et al., 2005). This type of

action is in contrast to nicotine which acts as a nicotine receptor agonist and incurs a strong and

short-lived dopamine release (Julien, 2005). Varenicline's slow release of dopamine is believed

to counteract nicotine-related withdrawal symptoms during abstinence (Coe et al., 2005). A

meta-analysis of varenicline's cessation success was recently conducted and at standard dose

levels, varenicline increased the probability of successful abstinence 2 to 3 times compared to

unassisted quit attempts (Cahill, Stead, & Lancaster, 2011). In addition, more individuals

abstained successfully with varenicline compared to buproprion and among two open-label trials

versus nicotine replacement therapy, varenicline was observed to have a small benefit (Cahill et

al., 2011). These findings using a partial agonist suggest patterns of dopamine activation and

inactivation are important for maintaining and sustaining patterns of tobacco use.

The link between nicotine-induced neurobiological effects including dopamine system activation and patterns of tobacco use initiation has been addressed by many models. One model suggests that the first several drug use episodes cause a brief decrease in reward threshold (Koob & Le Moal, 1997; Koob, 1999). This decrease in reward threshold leads to the perception of events that are usually perceived as neutral to be perceived as pleasant, and events that are usually perceived as pleasant to be perceived as more pleasant. These drug-induced changes in the hedonic value of events increase the likelihood of subsequent or chronic drug self-administration (i.e., are positively reinforcing; Koob & Le Moal, 1997; Koob, 1999). Evidence supporting this model can be observed during examinations of the acute effects of nicotine administration. These acute effects, which include positive subjective and stimulant-like cardiovascular effects, may positively reward initial tobacco use episodes increasing the likelihood for chronic use. Chronic use of nicotine also produces neurobiological effects, some of which may be long-lasting neural adaptations, influencing the progression to tobacco dependence.

Negative reinforcement/dependence: cigarette smoking. While initial drug use episodes (i.e., cigarette smoking) are reinforced positively, chronic drug administration changes the organism's underlying neurobiology and may make negative reinforcement more relevant in explaining chronic drug use (Koob & Le Moal, 1997; Koob, 1999). For example, one study examined the long term changes in brain dopaminergic parameters and nicotinic receptors in response to smoking among autopsy samples from normal elderly individuals with an identified smoking status and a specific genotype (Court et al., 1998). Findings indicated dopamine

turnover was reduced and levels of dopamine receptors were unchanged in the smokers compared to age-matched non-smokers, despite the finding of increased numbers of high-affinity nicotine receptors observed in smokers' brain areas (hippocampus, cerebellum, and striatum). This group reported their findings (i.e., reduced turnover and increased nicotine receptors) as consistent with the attenuated efficacy of these receptors in smokers (Court et al., 1998). Results indicated the possibility that chronic cigarette smoking was associated with a reduction of dopaminergic neuron firing in the nigrostriatal region of the brain (Court et al., 1998). According to one model, these and other changes in neurobiology may indicate an overall increase in the baseline reward threshold in chronic smokers (Koob & Le Moal, 1997; Koob, 1999).

This increased reward threshold is less apparent when the drug is being administered, but becomes more noticeable when the drug is not administered. In a drug dependent organism with an increased reward threshold and no access to drug, events that are usually perceived as neutral are perceived as unpleasant, and events that are usually perceived as pleasant are perceived as neutral (Koob & Le Moal, 1997; Koob, 1999). In terms of cigarette smoking (i.e., nicotine self-administration), chronic nicotine exposure may alter the dopamine system and thus may increase the smoker's reward threshold. This increased threshold becomes more apparent during periods of tobacco abstinence, when an aversive syndrome appears (e.g., Hughes & Hatsukami, 1986; Breland et al., 2002). Interestingly, according to the model, drug administration has the potential to decrease reward threshold temporarily, even in the dependent organism (Koob & Le Moal, 1997). When the reward threshold is reduced, the perception of events is altered (i.e., events

perceived as unpleasant are perceived as neutral or pleasant), and this drug-induced alteration of events previously perceived as unpleasant increases the likelihood of subsequent drug self-administration. In this context, these drug administrations in the dependent organism are maintained via negative reinforcement (e.g., Eissenberg, 2004).

In summary, the positive and negative reinforcing effects of tobacco-delivered nicotine may have a direct influence on the initiation and maintenance of cigarette smoking. These effects also may be relevant to an alternative tobacco use method that is gaining popularity in the U.S.: waterpipe tobacco smoking.

Nicotine's role in other forms of tobacco use: waterpipe tobacco smoking. While adult cigarette smoking levels in the U.S. are at their lowest in the past twenty years (19.8%; CDC, 2008), recent survey results indicate that the prevalence of an alternative form of tobacco use, waterpipe tobacco smoking, may be increasing among U.S. adolescents and young adults (Eissenberg, Ward, Smith-Simone, & Maziak, 2008; Smith-Simone, Maziak, Ward, & Eissenberg, 2008; Primack et al., 2008; Sutfin et al., 2011). Interestingly, this method of smoking is centuries old and has links to the countries of southwest Asia (e.g., Lebanon, Syria; see El-Roueiheb et al., 2008; Ward et al., 2006) and north Africa (Egypt; El-Setouhy et al., 2008). Tobacco waterpipes most often seen in the U.S. have a fired-clay head, metal body, glass or acrylic water bowl, and leather or plastic hose (see Figure 1). The bowl is partially filled with water and the head is filled with waterpipe tobacco (sweetened and flavored tobacco) upon which a lit piece of charcoal is placed (separated from the tobacco by perforated aluminum foil). Charcoal is necessary to heat the moistened waterpipe tobacco sufficiently to produce smoke
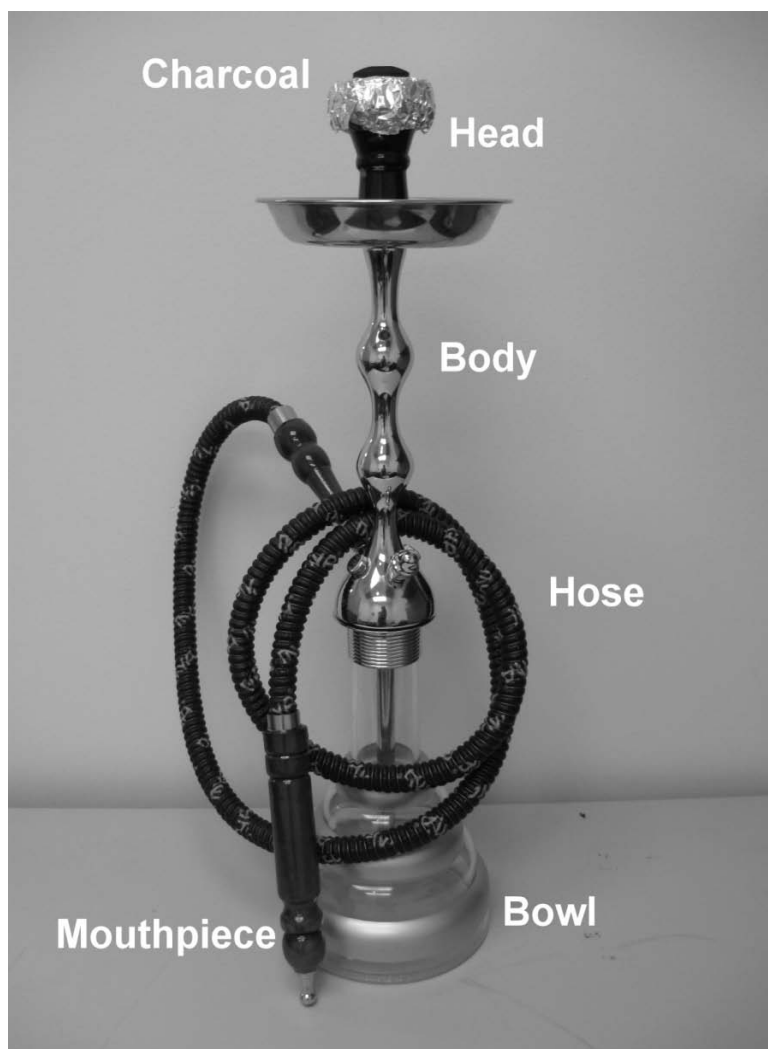
Figure 1. Typical waterpipe apparatus. A waterpipe prepared for tobacco smoking, including perforated foil separating the charcoal from the tobacco that has been placed in the head.

when the smoker inhales and draws air over the burning charcoal. The smoke then travels

through the waterpipe, the water, and finally into the hose to the user (Shihadeh, 2003). Among

U.S. college students, prevalence estimates of past 30-day waterpipe tobacco smoking range

from 10-20% (Eissenberg et al., 2008; Primack et al., 2008; Sutfin et al., 2011). This U.S.

prevalence estimate is surprisingly high for a tobacco smoking method associated with the

southwest Asia and north Africa. Indeed, waterpipe tobacco smoking appears to be spreading

globally (e.g., Maziak, Ward, Soweid, & Eissenberg, 2004; WHO, 2005; Cobb, Ward, Maziak,

Shihadeh, & Eissenberg, 2010), and this spread is likely due to several factors..

Factors Contributing to the Global Spread of Waterpipe Tobacco Smoking

Several factors may be contributing to the global spread of waterpipe tobacco smoking,

including the perception that waterpipe smoke is less dangerous than cigarette smoke, the easy

access to flavored waterpipe tobacco, and the fact that waterpipes deliver the reinforcing

stimulant drug nicotine.

Misconceptions regarding waterpipe tobacco smoking. One factor that may be

contributing to the global spread of waterpipe tobacco smoking is the perception that waterpipe

smoke is less dangerous than cigarette smoke. This perception has been reported in virtually

every survey of waterpipe tobacco smokers, from Syria (Maziak, Eissenberg, et al., 2004) to

England (Jackson & Aveyard, 2008) to Canada (Roskin & Aveyard, 2009) and the U.S. (Smith-

Simone et al., 2008; Smith, Curbow, & Stillman, 2007). Interestingly, studies that have

examined waterpipe smoke toxicant content do not support this perception: like cigarette smoke,

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waterpipe smoke contains nicotine, carbon monoxide (CO), and "tar" containing potent

carcinogens (Shihadeh, 2003; Shihadeh & Saleh, 2005; Maziak et al., 2009). Moreover,

waterpipe smoking behavior (i.e., "topography") is dramatically different than cigarette

topography, such that, during a typical 45-minute waterpipe tobacco smoking episode, the

smoker inhales 50-100 times more smoke than during a 5-minute cigarette smoking episode (see

Table 1; Cobb et al., 2011). Cross-study comparisons indicate that, relative to a single cigarette,

the smoke produced by a 45-minute waterpipe tobacco smoking episode contains 1.7 times the

nicotine, 6.5 times the CO, and 46 times the "tar" (Shihadeh & Saleh, 2005; Djordjevic,

Stellman, & Zang, 2000). Taken in sum, these data concerning waterpipe tobacco smoke

toxicant content and smoker topography suggest that waterpipe smoke is not less dangerous than

cigarette smoke, and support growing public health concern and calls for more research

regarding this tobacco use method (e.g., Maziak, 2008; El-Nachef & Hammond, 2008; Klein,

2008; WHO, 2005).

Easy access to flavored waterpipe tobacco. Another factor that may contribute to the

global spread of waterpipe use involves easy access of waterpipe products (via the internet with

little regulation or age verification) that include sweetened and flavored waterpipe tobacco.

Tobacco used for waterpipe tobacco smoking is distinctly different than the types and

preparations found in cigarettes or other smokeless tobacco products (see Figure 2). Although

other forms exist, the most popular type of waterpipe tobacco preparation is known as ma'assel

(i.e., "honeyed" tobacco; Knishkowy & Amitai, 2005; Shihadeh, 2003). Ma'assel preparations

Table 1.

Mean puff topography for waterpipe users and cigarette users

Topography variable	Waterpipe		Cigarette	
	N = 61[1]	N = 52[2]	N =30[3]	N = 56[4]
Puff number	169.0	171.0	10.0	12.7
Puff volume (ml)	511.0	530.0	51.0	48.6
Puff duration (s)	3.21	2.6	1.4	1.5
Interpuff interval (s)	12.6	15.5	30.7	21.3

[1] Maziak et al., 2009
[2] Shihadeh, Azar, Antonios, & Haddad, 2004
[3] Breland et al., 2006
[4] Djordjevic et al., 2000.

Figure 2. Ma'assel waterpipe tobacco products. Panel A displays a variety of ma'assel waterpipe tobacco products. Panel B displays a ma'assel non-fruit flavor: X on the Beach. Panel C is a typical label for ma'assel tobacco displaying ingredients. Panel D shows a waterpipe head loaded with ma'assel with perforated foil, ready for tobacco smoking. Panel E is a close-up of a typical ma'assel tobacco preparation.

contain approximately 30% tobacco and 70% honey, fruit, or molasses as well as sweeteners and flavorings (Knishkowy & Amitai, 2005).This blend of ingredients makes the waterpipe preparations very moist. The moistness of the product is one reason charcoal or another heat source must be used to the heat waterpipe tobacco to produce smoke. The variety of flavors of waterpipe tobacco is virtually limitless: there are fruit flavors (e.g., apple, peach, strawberry, kiwi, watermelon), candy flavors (e.g., bubble gum, chocolate, licorice), alcohol flavors (e.g., pina colada, apple martini, margarita), and others (e.g., kashmir, red tea, jasmine, cloves). In one survey of 201 U.S. waterpipe tobacco smokers, 80% cited flavor as one of the reasons that they smoke tobacco in a waterpipe (Smith-Simone et al., 2008). Among 31 regular waterpipe smoking participants recruited for a laboratory study of waterpipe smoking in the U.S., fruit flavors were most commonly preferred to smoke during their "favorite" brand session (Eissenberg & Shihadeh, 2009). Flavored waterpipe tobacco availability has been cited as important in understanding the increase in waterpipe tobacco smoking in Syria (Rastam, Ward, Eissenberg, & Maziak, 2004) and may also be relevant to the U.S. Alarmingly, adolescents and young adults in the U.S. can buy waterpipe tobacco and other supplies easily over the internet without age verification. Many companies based in the Eastern Mediterranean Region manufacture and import the specialized tobacco, including Al Fakher, Al Waha, Nakhla, Romman, and Fumari (American Lung Association, 2007). In addition, some companies use distributors based in the U.S. in order to sell their products more easily, and several U.S. companies manufacture and distribute their own brands of the waterpipe tobacco (e.g., Tangiers Tobacco LTD, San Diego, CA; Sahara Smoke Company, Statesboro, GA). Taken together, easy

access to a wide variety of flavored waterpipe tobacco may be another factor contributing to the spread of waterpipe tobacco smoking to this country.

Waterpipe tobacco and nicotine. Cigarettes deliver physiologically active nicotine doses (Henningfield, Miyasato, & Jasinski, 1985) and these doses of this stimulant drug are thought to reinforce subsequent cigarette smoking (e.g., Glautier, 2004; Eissenberg, 2004). Several studies suggest that waterpipes also deliver physiologically active nicotine doses. One of the earliest studies to examine waterpipe smoke composition was performed in Lebanon (Shihadeh, 2003). This study incorporated the use of a smoking machine powered by a vacuum pump attached to a waterpipe loaded with 10 grams of a common waterpipe tobacco. The machine replicated a standard smoking pattern of 100 puffs of 300 milliliters (ml) that were 3 seconds long and separated by 30 seconds. Results indicated an average nicotine yield of 2.25 mg nicotine from the smoke produced during the entire session (Shihadeh, 2003). In comparison, an average nicotine yield for a low-yield cigarette was 1.74 mg (Djordjevic et al., 2000). Unfortunately, the waterpipe toxicant results (Shihadeh, 2003) are limited in that they only report what is present in the smoke and not what is delivered to the smoker. A clinical study that addresses this issue involved 14 men who regularly ($\geq$ 3 times per week) smoked tobacco using a waterpipe and examined nicotine levels in saliva, urine, and plasma before and after a 45-minute waterpipe tobacco smoking episode (Shafagoj, Mohammed, & Hadidi, 2002). Prior to the smoking session, participants were required to abstain from tobacco use for at least 84 hours. The mean plasma nicotine level prior to smoking was 1.11 ng/ml (SD = 0.62) and after smoking was 60.31 ng/ml (SD = 7.58 ng/ml). The mean saliva nicotine level rose from

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1.05 ng/ml (SD = 0.72) to 624.74 ng/ml (SD = 149.3) ng/ml after smoking. The mean amount of

nicotine excreted during the 24-hour urine collection following smoking was equal to

approximately 73.59 µg (SD = 18.28; Shafagoj et al., 2002). In further support of waterpipe

tobacco smoking's ability to deliver nicotine, a recent meta-analysis of six waterpipe studies that

measured nicotine or cotinine levels in human participants suggested that daily use of a

waterpipe to smoke tobacco produced a 24-hr urinary cotinine level equivalent to that produced

by smoking 10 cigarettes/day (Neergaard, Singh, Job, & Montgomery, 2007). However, until

recently, there have been no published placebo controlled studies demonstrating conclusively

that waterpipe tobacco smokers are exposed to physiologically active nicotine doses.

One study performed at VCU has addressed this gap in the literature concerning the

nicotine-related physiological effects with waterpipe tobacco smoking (Blank et al., 2011). This

study was a within-subject, double-blind, randomized design that included thirty-seven

participants who were occasional waterpipe smokers (2-5 waterpipes per month; Blank et al.,

2011). In each condition participants smoked a waterpipe loaded with 10 grams of a normally-

marketed waterpipe product. In one condition the product was the participant's preferred flavor

of waterpipe tobacco, and in the other, the product was the same flavor of a non-tobacco

preparation marketed for use in waterpipes (see www.soex.com/herbalmolasses.html). Results

demonstrated clearly that, under these conditions, waterpipe tobacco smokers are exposed to

nicotine (mean plasma increase=3.6 ng/ml, standard error of mean [SEM]= 0.7). In addition, the

results indicated that this exposure increased as the duration of smoking increased, and that the

nicotine was physiologically active. During the tobacco session, the mean ($\pm$ SEM) heart rate

increase was 8.6 ± 1.4 bpm while during the non-tobacco the mean heart rate increase was 1.3 ± 0.9 bpm (Blank et al., 2011).

Another study reveals waterpipe tobacco smoking-associated subjective effects (Maziak et al., 2009). Sixty-one waterpipe tobacco smokers, who were overnight tobacco abstinent, were asked to smoke a waterpipe *ad libitum* in a laboratory using their preferred waterpipe tobacco brand and flavor (all participants used a variety of ma'assel). Participants' CO levels and subjective measures were assessed prior to and after waterpipe tobacco smoking. Subjective questionnaires included VAS items assessing tobacco-related abstinence symptoms (e.g., "urges to smoke", "irritability") and other nicotine-related items (e.g., "sweaty", "nauseous", "dizzy", "light-headed"). Significant post-smoking decreases were observed for multiple tobacco abstinence measures (i.e., "urges to smoke") and significant post-smoking increases were observed for several items associated with the nicotine exposure (i.e., "dizzy", "light-headed"). The results observed in this study support the notion that waterpipe tobacco smoking may relieve nicotine abstinence-related effects as well as induce direct effects associated with nicotine administration (Maziak et al., 2009).

The three factors discussed above, misperceptions of waterpipe tobacco smoking, access to flavored tobacco, and nicotine exposure, may be contributing the global increase in waterpipe tobacco smoking. Another factor that may contribute to the reinforcing effects of waterpipe tobacco smoking is the co-administration of another mild psychomotor stimulant: caffeine.

Caffeine's Pharmacokinetics and Pharmacodynamics

Caffeine is the most commonly consumed psychoactive substance in the world (Nehlig, Daval, & Debry, 1992) and approximately 80% of the U.S. population consumes caffeine daily in some form (Hughes, Oliveto, Bickel, Higgins, & Badger, 1993). In contrast to the tobacco-related health effects of nicotine, caffeine is associated with few known health risks and low abuse liability (Daly & Fredholm, 1998).

Generally, caffeine is classified a psychomotor stimulant and is consumed usually in beverages. In humans, orally ingested caffeine is rapidly absorbed from the gastrointestinal tract and increased blood plasma levels are reached within 30 to 45 minutes; complete absorption occurs over the next 90 minutes (Newton et al., 1981; Fredholm, Battig, Holmen, Nehlig, & Zvartau, 1999). The half-life of caffeine is about 3.5 to 5 hours in most people (Newton et al., 1981; Julien, 2005, p. 227). Once absorbed, caffeine produces stimulant-like effects in the central nervous system as well as cardiac, respiratory, and diuretic effects. Common behavioral effects in humans include descriptions of enhanced alertness, energy, wakefulness, and fatigue reduction (see Daly & Fredholm, 1998; Griffiths & Mumford, 1996), but these effects are often dose-dependent. Large doses of caffeine can cause adverse symptoms such as agitation, anxiety, tremors, rapid breathing, and insomnia (Ashton, 1987; Chait, 1992; Schuh & Griffiths, 1997; Griffiths & Mumford, 1996). This diverse array of effects may be associated with caffeine's different mechanisms of action at high versus low doses (Daly & Fredholm, 1998; Garrett & Griffiths, 1997).

In doses regularly achieved by humans, caffeine's primary mechanism of action is as a competitive adenosine receptor antagonist (Nehlig et al., 1992; Daly & Fredholm, 1998). Adenosine is a neuromodulator that stimulates the release some neurotransmitters in the CNS; it also acts as a vasodilator and can exert sedative, depressant, and anticonvulsant actions (Eltzschig, 2009; Hasko, Linden, Cronstein, & Pacher, 2008; Julien, 2005, p. 229). Currently, there are four common adenosine receptors denoted A_1, A_{2A}, A_{2B}, and A_3 (Jacobson, 2009). By blocking adenosine receptors, primarily A_1 and A_{2A} (G-protein coupled), caffeine's effects are often described as opposite to those of adenosine. Adenosine A_1 receptors are present in almost all brain areas (Goodman & Snyder, 1982; Fastbom, Pazos, Probst, & Palacios, 1987) and appear to be present on cell bodies as well as nerve terminals. Adenosine A_{2A} receptors are more strongly expressed in the striatum, nucleus accumbens, and tuberculum olfactorium (Fredholm et al., 1999; Parkinson & Fredholm, 1990; Schiffmann et al., 2003; Schiffmann, Fisone, Moresco, Cunha, & Ferré, 2007). Specifically in the striatum, there is evidence of a colocalization of A_{2A} and D_2 receptors (Svenningsson et al., 1997) and A_1 receptors and D_1 and D_2 receptors (Ferre et al., 1996; Agnati, Ferre, Lluis, Franco, & Fuxe, 2003; Franco et al., 2007). This co-expression may account for caffeine's mediation of neurotransmitter release including dopamine within this system (Ferre, 2008; Latini, Pazzagli, Pepeu, & Pedata, 1996). Also in support of this hypothesis is the finding that some effects of caffeine can be blocked by selective D_1 and D_2 antagonists, and caffeine can also potentiate effects produced by indirectly acting dopamine receptor agonists like amphetamine (Schechter, 1977) and cocaine (Misra, Vadlamani, & Pontani, 1986). These results suggest that caffeine administration may activate dopaminergic systems indirectly.

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There is still debate as to whether other unknown receptor systems also may be responsible for caffeine's array of effects (Daly & Fredholm, 1998). Chronic caffeine exposure results in the development of tolerance to many acute effects including physiological, subjective, and behavioral effects (see Griffiths & Mumford, 1996). This tolerance may be associated with the alteration of receptor densities (e.g., up regulation of the A_1 adenosine receptors), which occurs in response to chronic caffeine administration (Ferré, 2008; Johansson, Georgiev, Lindström, & Fredholm, 1997). Taken together, caffeine's mechanism of action both acutely and chronically may account for the various effects associated with its use.

Acute Effects of Caffeine

Similar to nicotine, caffeine is a stimulant drug that users self-administer, and the acute physiological and subjective effects associated with caffeine administration may act as a positive reinforcer for subsequent use (Smith, 2002; Garrett & Griffiths, 1997). Like other drugs that act as positive reinforcers, caffeine administration is associated modestly with dopamine system activation. There is debate to whether caffeine induces dopamine concentration increases in areas usually associated with drugs of abuse (i.e., nucleus accumbens, VTA, striatum), but there is evidence of dopamine increase in some areas of the brain following caffeine administration (Solinas et al., 2002; Acquas, Tanda, & Di Chiara, 2002). Some believe the mechanism for dopamine release associated with caffeine administration is due to both presynaptic and postsynaptic activity in the striatum, due to the presence of A_1 and A_{2A} receptors in both areas as well as their colocalization with dopamine receptors (D_1 and D_2; Ferré, 2008).

Preclinical research has demonstrated some areas of the brain are associated with dopamine concentration increases following acute caffeine administration. One study examined the effects of caffeine on extracellular dopamine in freely moving rats implanted with probes in the nucleus accumbens shell and core and in the medial prefrontal cortex (Acquas et al., 2002). This study included five caffeine doses (0.25, 0.5, 1.0, 2.5, and 5.0 mg/kg) administered i.v. and four caffeine doses (1.5, 3, 10, and 30 mg/kg) administered intraperitoneally (i.p.). For the caffeine doses administered i.v., researchers observed dose dependent dopamine concentration increases in the medial prefrontal cortex while levels in the nucleus accumbens were unaffected. These concentration peak effects appeared 10, 20, or 30 minutes after administration depending on dose. The dopamine concentrations after the i.p. caffeine doses were tested only in the nucleus accumbens shell and core and produced no significant changes in dopamine concentrations. The effects of i.v. administered caffeine (i.e., dopamine increases in prefrontal cortex and none in the nucleus accumbens) were duplicated after intravenously administering doses of either an adenosine A_1 receptor antagonist or A_{2A} receptor antagonist (Acquas et al., 2002). A similarly designed study which examined the effects of four doses of caffeine (3, 10, 30 and 100 mg/kg) delivered i.p. on dopamine concentrations in the nucleus accumbens shell and core reported contrasting results (Solinas et al., 2002). In the shell of the nucleus accumbens, 10 and 30 mg/kg caffeine doses were observed to produce significant increases in extracellular concentrations of dopamine, but at other caffeine doses, effects were not significantly different than saline. In the core of the nucleus accumbens, only the 30 mg/kg dose of caffeine produced a significant increase in dopamine levels, but this effect was lower and significantly different than

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the increase observed in the nucleus accumbens shell. The effects in the nucleus accumbens

shell were duplicated after intravenously administering doses of an adenosine A_1 receptor

antagonist, but not after administering an A_{2A} receptor antagonist (Solinas et al., 2002). Both of

these findings differ from those of nicotine, which unequivocally increases dopamine

concentrations in nucleus accumbens core and shell (see Ponteri, Tanda, Orzi, & Di Chiara,

1996; Watkins, Koob, & Markou, 2000) via stimulation of dopamine releasing neurons.

Importantly, an absence of dopamine release in the either the nucleus accumbens shell or core,

which is linked to the reinforcement and reward properties of many drugs of abuse, does not

imply that caffeine lacks these actions.

Some clinical research suggests that caffeine has acute positive effects that reinforce use.

One early survey revealed that coffee drinkers report having coffee in the morning because of its

pleasant taste and stimulating effects, and heavy ($\geq$5 cups per day) coffee drinkers were more

likely to report these reasons (Goldstein & Kaizer, 1969). In addition, more heavy coffee

drinkers emphasized the increased sense of well-being associated with coffee drinking, and cited

that coffee increased alertness and activity (Goldstein & Kaizer, 1969). Given that caffeine users

can identify several positive effects associated with coffee drinking, positive reinforcement may

be a factor influencing caffeine use.

Other more controlled clinical laboratory designs have examined the acute subjective

effects associated with caffeine administration (Bruce, Scott, Lader, & Marks, 1986; Garrett &

Griffiths, 2001; Childs & de Wit, 2006). In a recent study, 102 light caffeine users (< 300 mg

per week from diet) were recruited to participate in four double-blind laboratory sessions that

differed by caffeine dose administered via capsule (0, 50, 150, or 450 mg; Childs & de Wit, 2006). Participants were overnight caffeine abstinent prior to any session, and subjective measures included the POMS and VAS scaled items (e.g., "Do you feel any drug effect?", "Are you high?", and "stimulated"). Results indicated that relative to placebo the 450 mg dose significantly increased ratings of "feel drug" and "drug high" and both the 150 mg and 450 mg dose significantly increased ratings of "stimulated". The authors concluded that at the doses tested in this study, caffeine could produce some beneficial mood enhancing effects (Childs & de Wit, 2006). These results and other laboratory examinations of caffeine (see also Garrett & Griffiths, 2001; Bruce et al., 1986) indicate that caffeine may produce acute positive subjective effects in humans.

The clinical laboratory has also been used to demonstrate cardiovascular effects associated with acute caffeine administration. A placebo controlled within-subject examination of caffeine doses of 100, 300, and 400 mg delivered via capsules was performed among six participants who were at least daily coffee drinkers (Astrup et al., 1990). Participants abstained overnight from tobacco, food, and all caffeinated products. Outcome measures included heart rate and blood pressure. Relative to placebo, measures of heart rate after all doses of caffeine did not show any significant increases, but there was a trend for decreased heart rate following caffeine administration. For systolic and diastolic blood pressure, small increases were observed after the 100 and 200 mg caffeine doses, but these increases were not significantly different from placebo. In contrast, the 400 mg caffeine dose significantly increased systolic blood pressure and diastolic blood pressure (average increase for both was 6.5 mm Hg). Limiting these results

was the sample size and use of only one cardiovascular measurement per post-administration time point (Astrup et al., 1990). This study is a good example of common cardiovascular effects associated with acute caffeine administration (i.e., increased blood pressure and little change in heart rate), as well as common methodological issues (i.e., small sample size).

Two separate reviews of caffeine and cardiovascular-related effects literature support the notion that acute caffeine administration is associated with an increase in blood pressure and often little change in heart rate (Mort & Kruse, 2008; Nurminen, Niittynen, Korpela, & Vapaatalo, 1999). Among twenty studies that used a controlled design, random selection, and a population of normotensive participants, a single dose of caffeine of 200-250 mg (approximately 2-3 cups of coffee) on average produced an increase of 3-14 mm Hg systolic and 4-13 mm Hg diastolic pressure (Nurminen et al., 1999). Blood pressure elevations in response to caffeine were reported to occur within 30 minutes post-administration with maximal increases occurring 60-120 minutes after caffeine intake (Nurminen et al., 1999). In addition, this review indicated that acute caffeine administration may be associated with little change or a slight decrease in heart rate (Nurminen et al., 1999). Both literature reviews indicated that many factors may impact an individual's cardiac response to caffeine including dose, administration route, caffeine use history, and risk/history of hypertension (Mort & Kruse, 2008; Nurminen et al., 1999).

Overall, individual laboratory data and literature reviews examining the acute cardiovascular and subjective effects of caffeine indicate that caffeine administration may produce positive subjective effects, quantifiable increases in blood pressure, and little to no

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change in heart rate. These acute subjective and cardiovascular effects may impact the positive reinforcement-related effects of caffeine use.

Unlike some drugs of abuse (e.g., nicotine), evidence concerning caffeine's ability to produce reinforcing effects leaves many unconvinced despite numerous laboratory-based studies that have examined the acute reinforcing effects of caffeine (Griffiths & Woodson, 1988; Evans, Critchfield, & Griffiths, 1994). One double-blind design assessed the reinforcing effects of caffeine among twelve regular caffeine users (Griffiths & Woodson, 1988). Each caffeine condition consisted of a three day sequence where each experimental session day, participants orally ingested two color-coded capsules. One capsule always contained placebo and the other contained 0, 100, 200, 400, or 600 mg of caffeine. Two days were forced exposure days where participants received two different types of color-coded capsules (e.g., red then green), followed by a choice day. On the choice day, participants could choose the color of capsule they received. The exposure and choice procedure periods were repeated for each dose of caffeine. Subjective effect measures included scales from the POMS, mood-related items rated using a four-point scale (e.g., "alert/attentive/observant/able to concentrate", "active/stimulated/energetic", "jittery/nervous/shaky" headache"), and a general "liking" scale of capsule effects. Although the variability across participants was high, a significantly greater percentage of participants chose to receive caffeinated capsules after a pairing with 100 mg or 200 mg caffeine dose, and these choices diminished at the higher caffeine doses. Subjective effect data indicated that caffeine administration produced significant increases in ratings of "liking" and "active/stimulated/energetic" as well as significant decreases in ratings of

"sleepy/tired/drowsy/half-awake" and "headache". Higher doses of caffeine increased some of these subjective effects. Using the choice data for their basis, the authors of this study reported significant caffeine positive reinforcement was demonstrated in five of the twelve subjects at one or more caffeine doses (Griffiths & Woodson, 1988). Similarly, another study found that caffeine deprived and non-deprived individuals picked caffeine over a placebo in 80% of choice trials (Evans et al., 1994). Results from these studies suggest that on some level, caffeine does positively reinforce use.

In summary, the acute effects of caffeine include modest increases in dopamine release in some areas of the brain, positive subjective effects, predictable cardiovascular responses (i.e., increased blood pressure, decreased heart rate), and reinforcing effects. While acute caffeine effects may increase the likelihood of subsequent or chronic use of caffeine via positive reinforcement, like nicotine, chronic caffeine ingestion may impact users differently via tolerance and the suppression of abstinence-related symptomology (i.e., negative reinforcement).

Chronic Effects of Caffeine

Like many drugs of abuse (e.g., nicotine) caffeine is capable of producing patterns of habitual use. Chronic or heavy users of caffeine undergo neurobiological and behavioral changes in response to caffeine. As with the dependence model described earlier concerning nicotine, these changes in neurobiology may indicate an overall increase in the baseline reward threshold in chronic caffeine users (Koob & Le Moal, 1997; Koob, 1999). There is clear evidence that chronic use of caffeine even in daily doses is associated with tolerance (Griffiths, & Mumford, 1996), and discontinuation of caffeine use may produce an abstinence syndrome

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(Juliano & Griffiths, 2004). Caffeine abstinence symptoms include headache, fatigue, and negative mood states (Griffiths & Mumford, 1996). These symptoms often climax over the first 1 to 2 days following caffeine abstinence and tend to decrease within a few days (Daly & Fredholm, 1998). Re-administering caffeine often relieves caffeine withdrawal symptoms (Juliano & Griffiths, 2004)

The fact that caffeine consumption reverses the effects of abstinence suggests at least some of caffeine's reinforcing value is prevention of these negative symptoms (Juliano & Griffiths, 2004; Strain, Mumford, Silverman, & Griffiths, 1994). One study performed among eleven "caffeine dependent" individuals found strong evidence in support of this theory (Strain et al., 1994). These eleven participants were selected from a larger population using a structured clinical interview with classic drug dependence criteria taken from Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV; American Psychological Association, 1994). Post-screening, two double-blind caffeine-abstinence periods of two days in length were administered. Caffeine abstinence periods were achieved by asking participants to adhere to a strict diet for two days that restricted caffeine intake although these restrictions were given without reference to caffeine (Strain et al., 1994). Each day during the two-day periods, participants received capsules containing in random order, either caffeine (an amount equal to their individual average daily caffeine consumption) or placebo. Capsule administration times were spaced throughout the day to match the pattern of the individuals reported caffeine consumption (typically three times per day) and assessments occurred on the second day of each of the 2-day study periods. Assessments included the POMS and a checklist that assessed

symptoms related to caffeine abstinence (e.g., headache, drowsy/sleepy). Nine of the subjects showed evidence of caffeine abstinence symptomology during the placebo period and seven reported maximal ratings of headache during the day they received placebo. In addition, eight of the subjects reported functional impairment in normal daily activities during the placebo periods and almost half of the participants also reported use of an analgesic during the placebo period. There was only one report of functional impairment during the caffeine period (Strain et al., 1994). These results and others (Griffiths, Bigelow, & Liebson, 1986) suggest that caffeine alleviates the unpleasant symptoms associated with acute abstinence among chronic users.

Further support for caffeine's ability to act as a negative reinforcer in chronic users comes from a double-blind study where 48 moderate caffeine consumers were randomly assigned into two groups (caffeine maintenance or abstinence) for two weeks and entered an additional two week caffeine conditioning procedure (Tinley, Yeomans, & Durlach, 2003). Throughout the 4 week period participants were supplied with drinks (caffeinated or decaffeinated) that replaced their normal caffeinated tea and coffee consumption. During the final two week conditioning procedure, all participants attended four non-consecutive laboratory sessions where they evaluated a novel drink containing either 100 mg caffeine or placebo. Prior to each conditioning session, participants were asked to only drink water the night before to induce acute caffeine abstinence. During the conditioning sessions, participants were asked to rate the novel drink using VAS scales (e.g., "bitter", "sweet," and "pleasant"). Only acutely abstaining participants (i.e., the caffeine maintained group) showed an increase in rated drink pleasantness for the caffeinated drink during the conditioning phase. Chronically abstinent

participants (i.e., caffeine abstinence group) showed a decrease in ratings of drink pleasantness for the caffeinated drink and no change for the decaffeinated drink during conditioning. These data suggest that the ability of caffeine to reinforce changes in ratings of "pleasantness" or "flavor liking" are driven by the alleviation of abstinence symptoms among chronic caffeine consumers and not by the induction of positive effects (Tinley et al., 2003). These findings highlight caffeine's role as a negative reinforcer in regular caffeine users.

Another way of experimentally differentiating the positively reinforcing components from the negatively reinforcing components is to use a multiple-choice procedure in which participants choose between receiving a drug and receiving different amounts of money. Using this technique to determine how much money moderate caffeine users (mean = 379 mg/day caffeine) would sacrifice for caffeine, researchers reported that caffeine was worth an amount not significantly different from $0.00, and participants would forfeit on average $2.51 in order not to receive the placebo (and instead receive caffeine; Schuh & Griffiths, 1997). Those who received the placebo reported symptoms such as headaches and feeling worn out, and those who had more headaches and felt less alert were willing to sacrifice more money in order to avoid the placebo (Schuh & Griffiths, 1997). Based on these results, the authors concluded that caffeine consumption is motivated more by desire to avoid negative abstinence effects than by the positive effects of caffeine in moderate caffeine users.

The previous two sections demonstrate that, like nicotine, caffeine offers positive and negative reinforcement which according to some models of drug abuse may increase the likelihood for subsequent use (Koob & Le Moal, 1997; Koob, 1999). Interestingly, the

rewarding/reinforcing aspects of nicotine and caffeine may also act in combination as they are commonly co-administered. In a review of six epidemiological studies, over 86% of cigarette smokers reported daily caffeine use versus 77% of non-smokers (Swanson et al., 1994). A laboratory study of 40 smokers and 40 non-smokers matched on multiple characteristics including age, gender, race, and body mass index found an even larger difference: 95% of smokers used caffeine versus 75% of non-smokers ($p < 0.05$; Zhang, Samet, Caffo, Bankman, & Punjabi, 2008). Empirical evidence also indicates that the interaction of these two drugs may produce an enhancement of rewarding or pleasurable effects that could impact patterns of tobacco use initiation and maintenance.

Interactions Concerning Nicotine and Caffeine

Drugs may interact on many levels within an organism. Already well-defined is the finding that the metabolism of caffeine is greatly enhanced in tobacco users (Kroon, 2007; Zevin & Benowitz, 1999; Kalow & Tang, 1991). This increased clearance of caffeine may contribute to the higher volumes of caffeine ingested by tobacco users compared to non-smokers (Swanson et al., 1994), but it may not account completely for the higher levels of overall use between smokers and non-smokers (Swanson et al., 1994; Zhang et al., 2008). Potentially, tobacco (i.e., nicotine administration) and caffeine may be interacting at another site that produces enhanced reward/reinforcement-related effects.

Sophisticated models exist to examine drug interactions in the realm of behavioral pharmacology, and there is often some confusion over the terms and definitions used to describe results obtained (Mitchell, 1976; Wessinger, 1986; Hertzberg & MacDonell, 2002). One means

of understanding types of drug interactions is by first classifying the relationship between the two drugs and the variable of interest into either a hetergic or homergic category (Wessinger, 1986). Hetergic drugs are two drugs that do not have similar effects; one drug produces the effect measured, and the other does not (e.g., consider two drugs that have opposite effects on heart rate). Homergic drugs are considered drugs that both produce the effects measured (e.g., consider caffeine and nicotine's positive reinforcement-related subjective effects). Two proposed models to examine homergic drugs are the effect-addition model and dose-addition model (Wessinger, 1986). The simplistic effect-addition model proposes that the combination of each drug of interest will produce an effect equal to the sum of each effect alone (i.e., effective-additive effect). Terms to describe results that are not equal to sum of each individual effect include "greater than effect-additive" or "less than effect-additive". These terms indicate results that are greater than the individual effects combined or less than the individual effects combined. The second model, the dose-addition model, incorporates the influences of dose and effect and examines the magnitude of effect a specific dose as a measure of drug potency (Wessinger, 1986). Both drugs of interest are considered different forms of the same substance with different potencies affecting the variable of interest (Wessinger, 1986). Dose response testing of each drug is necessary in order to utilize the dose-addition model. Although the dose-addition model may provide more information concerning the nature and magnitude of potential drug interactions than the effect-addition model, dose-addition is not used frequently among researchers examining the effects of nicotine and caffeine. Much of the research concerning these two drugs uses a small range or discrete drug doses and either compares the individual

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effects of each drug versus the combination or a variation of this method (see Perkins et al., 1994; Rose & Behm, 1991). These methods appear to lend themselves to the effect-addition model. Importantly, among clinical studies that evaluate marketed products (e.g., cigarettes and coffee) testing a complete dose response curve to utilize the more precise dose-addition model may be less feasible.

Of the empirical research examining the potential interaction of nicotine and caffeine, some evidence indicates caffeine can enhance some of nicotine's effects in preclinical models (Cohen, Welzl, & Battig, 1991; Gasior, Jaszyna, Peters, & Goldberg, 2000) and in humans. In clinical models, enhancement has been observed for some reward/reinforcement-related subjective effects (Perkins et al., 1994; Jones & Griffiths, 2003) and cardiovascular effects (Ray, Nellis, Brady, & Foltin, 1986; Smits, Temme, & Thien, 1993; Perkins et al., 1994). In addition, contradictory results have also been observed in smokers (e.g., attenuation of nicotine's subjective effects by caffeine, Rose & Behm, 1991; no effect of caffeine pretreatment upon nicotine-associated discriminative stimulus, subjective, or reinforcing effects; Perkins, Fonte, Stolinski, Blackesley-Ball, & Wilson, 2005), and among a group of non-smokers, nicotine's effects were not influenced by co-administration of a variety of caffeine doses (Blank, Kleykamp, Jennings, & Eissenberg, 2007). This growing body of literature demonstrates that caffeine and nicotine may interact on some measures including the enhancement of reward/reinforcement-related effects. Examinations of both preclinical and clinical laboratory work highlight important study design considerations when investigating the interaction of these two drugs.

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Preclinical Evaluations of Nicotine and Caffeine

Over the past few decades, many preclinical studies have examined the individual and combined effects of nicotine and caffeine. There is some evidence to support that acute caffeine administration can enhance the effects of nicotine that are related to its abuse potential. Acute pre-session co-administration of nicotine and caffeine has produced increases of locomotor activity (Cohen et al., 1991) and schedule-controlled behavior (White, 1988) under some conditions in rats. Chronic exposure to caffeine (which may more closely approximate human self-administration patterns) paired with nicotine has also been observed to facilitate the acquisition of nicotine self-administration (Shoaib, Swanner, Yasar, & Goldberg, 1999) and nicotine discrimination (Gasior et al., 2000; 2002) under certain conditions in rats. One mechanism used to explain these findings involves caffeine- induced dopamine release in tandem with nicotine-induced dopamine release (Garrett & Holtzman, 1994; Tanda & Goldberg, 2000), which may increase the likelihood of subsequent drug self-administration via the mechanism of positive reinforcement (Garrett & Griffiths, 1997).

Acute pre-treatment with caffeine is one means to examine how caffeine and nicotine interact. One preclinical study measured locomotor activity levels after acute nicotine and caffeine pretreatment in nicotine tolerant and nicotine naïve rats within a tunnel maze (Cohen et al., 1991). After two 6-minute trials in a tunnel maze, animals from each pre-treatment group (nicotine tolerant and nicotine naïve) were given a subcutaneous (s.c.) injection of saline, nicotine (0. 2 mg/kg), caffeine (8 mg/kg) or nicotine (0.2 mg/kg) and caffeine (8 mg/kg). In the nicotine naïve rats, nicotine exposure significantly decreased locomotor activity, which is a

common effect of nicotine administration. Interestingly, this decrease was not observed when nicotine and caffeine were administered simultaneously in the nicotine naïve rats, and in these animals this level of locomotor activity was not significantly different from saline. Among the nicotine tolerant rats, caffeine and nicotine in combination significantly increased locomotor activity above the saline level, but individually nicotine and caffeine had a small, not significant locomotor increase (Cohen et al., 1991). These results highlight caffeine's ability to influence some of the behavioral effects of nicotine, despite nicotine tolerance.

Chronic dosing with caffeine is another means to examine the interaction of caffeine and nicotine. A commons means to achieve chronic dosing among rodents is to add caffeine to their drinking water. One study that used this paradigm examined changes in ambulatory activity and discriminative stimulus effects to a variety of psychostimulants including nicotine (Gasior et al., 2000). Three groups of drug naive rats were randomly assigned to receive either tap water, a solution with 0.25/ml caffeine, or a solution with 1.0 mg/ml caffeine throughout the experiment. Rats were exposed to their solutions for at least one week before testing. Nicotine discrimination was trained with 0.4 mg/kg of nicotine delivered s.c. Exposure to the lower concentration of caffeine enhanced the stimulatory effects of nicotine and did not produce tolerance to the acute stimulatory effects of caffeine, while the higher caffeine concentration did not alter the effects of nicotine on ambulatory behaviors, and resulted in the development of complete tolerance to the acute stimulatory effects of caffeine. In the nicotine discrimination paradigm, rats exposed to the lower caffeine concentration acquired the nicotine discrimination significantly faster than the placebo (tap water) animals, while the animals exposed to the higher concentration did not. In

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summary, pretreatment with the lower dose of caffeine enhanced the stimulatory and discriminative stimulus effects of nicotine while the higher dose had little effect of nicotine's behavioral effects and produced tolerance to caffeine's acute stimulatory effects (Gasior et al., 2000). This study indicates that caffeine dose may be an important determinant of the behavioral effects observed in response to nicotine co-administration.

A later study by the same group of researchers used a similar continuous dosing procedure to examine the effects of chronic dosing in combination with acute caffeine dosing prior to nicotine discrimination (Gasior et al., 2002). Two groups of rats (drinking solution: tap water or 1.0 mg/ml caffeine) were trained to discriminate 0.4 mg/kg nicotine s.c. from saline. A range of caffeine doses (1.0-30.0 mg/kg) were administered i.p. 15 minutes prior to discrimination testing. These doses produced an enhancement of the discriminative-stimulus effects of the threshold dose of nicotine (0.05 mg/kg) in the placebo (tap water) and caffeine pretreatment animals (Gasior et al., 2002). Chronic caffeine pretreatment did not significantly alter the responses observed with acute caffeine in combination with nicotine. The results, while complex, support the idea that caffeine interacts with the discriminative effects of nicotine. In contrast, another group performed a similarly designed study (Justinova et al. 2009), and did not observe caffeine's enhancement of nicotine's discriminative properties. If caffeine use enhances nicotine's discriminative stimulus properties, this action may intensify other reward or reinforcement-related properties of nicotine such that co-administration contributes to tobacco use initiation and maintenance.

In further support of this notion, one study has examined the effects of chronic caffeine exposure on i.v. nicotine self-administration (Shoaib et al., 1999). After catheter implantation surgery, rats were assigned to one of three groups: 1) access to tap water at all times, 2) access to caffeinated drinking water 7 days prior and for the first 14 days of nicotine self-administration, or 3) access to tap waterpipe before and during the first 14 days of nicotine self-administration. After the first 14 days, caffeine was either added to or removed from the drinking water for the last two groups to achieve the double cross-over design. Rats consumed on average 150-180 mg/kg of caffeine per day and following the first 7 days of exposure to tap water or caffeine water, rats were allowed to administer nicotine (0.03 mg/kg per infusion) in daily sessions. Results indicated that animals maintained on caffeinated drinking water acquired nicotine self-administration more rapidly than tap water maintained animals and a greater percentage of animals met the acquisition criteria. Furthermore, this caffeine-related enhancement of nicotine's reinforcing effects was specific; during extinction, the speed and final levels of extinction responding for caffeine and the tap water maintained animals were similar (Shoaib et al., 1999).

Some researchers have described the mechanism of action concerning a caffeine-induced enhancement of nicotine's effects as dopaminergically based (Garrett & Griffiths, 1997). If accurate, this premise would further support caffeine's possible augmentation of reinforcement-related properties of nicotine. Few researchers have examined dopamine concentrations after nicotine administration in rats chronically exposed to caffeine. One examination reported within a literature review of caffeine and nicotine's effects (Tanda & Goldberg, 1994) describes the

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results when group of rats received caffeine (3 mg/ml) in their drinking water for three weeks and control rats drank plain tap water (Tanda & Goldberg, 2000). In all animals s.c. nicotine administrations (0.2 and 0.4 mg/kg) produced a significant dose-related increase in dopamine levels in the shell of the NA. This effect was significantly enhanced in caffeine drinking rats relative to the control (tap water) rats. Although the mechanism of action is unclear, the effects observed during this study provide support for the role of enhanced dopamine transmission in the nucleus accumbens shell during concurrent caffeine and nicotine exposure (Tanda & Goldberg, 2000). If the co-administration of nicotine and caffeine produce this enhancement in humans as it does in rats, their use may affect patterns of tobacco use behavior.

While some preclinical studies provide support for the premise of caffeine-related enhancement of nicotine's effects, clinical studies may better approximate realistic human behavior patterns. Not surprisingly, the issue of nicotine/caffeine interactions in humans has received some empirical attention.

Clinical Laboratory Evaluations of Nicotine and Caffeine

Several clinical laboratory investigations among smokers and non-smokers have examined the effects of concurrent nicotine and caffeine administration. Many of these evaluations offer more uncertainty concerning the combined effects of these two drugs, as caffeine/nicotine dose, caffeine use history, and study design vary greatly across studies, but some results suggest that nicotine and caffeine may produce enhanced cardiovascular and subjective effects related to reward and reinforcement. These effects, if present, may impact patterns of tobacco use initiation and maintenance.

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An early naturalistic study examined the relationship between cigarette smoking and caffeine administration (Emurian, Nellis, Brady, & Ray, 1982). In this study, eight participants who smoked cigarettes and drank coffee resided in a controlled laboratory environment for 7-12 days with their own brand cigarettes and caffeinated coffee available constantly (Emurian et al., 1982). Results revealed a relationship between cigarette smoking and coffee drinking. A coffee drinking event tended to occur late in the inter-cigarette interval, and cigarette smoking was most likely during the 20 minutes immediately following coffee drinking. Also, an examination of the frequency of smoking and coffee drinking revealed a significant positive correlation between coffee drinking and smoking, thus those who smoked more cigarettes throughout the day also tended to drink more coffee (Emurian et al., 1982). This study was limited by the absence of a control group who drank de-caffeinated coffee. Determining whether caffeine, the act of coffee drinking, or some combination of the two was time-related to cigarette (i.e., nicotine administration) smoking is difficult. Nonetheless, this study provided impetus for determining whether smoking and coffee drinking was merely a coincidental pairing.

Clinical examinations that are more systematic provide better support for the relationship between nicotine and caffeine use. For example, investigators in another early study measured the effects of nicotine and caffeine on multiple measures including cardiovascular effects (Ray et al., 1986). Nine cigarette smokers who also drank coffee participated in four sessions corresponding to decaffeinated coffee only, decaffeinated coffee with own brand cigarette smoking, caffeinated coffee (4 mg/kg) only, and caffeinated coffee (4 mg/kg) with cigarette smoking. Participants were required to be overnight abstinent from nicotine and caffeine prior to

each session. Results indicated that the administration of caffeinated coffee with cigarette smoking increased blood pressure levels higher than cigarette smoking with decaffeinated coffee and caffeinated coffee alone. Although these results supported an enhancement of nicotine's effects by caffeine, the lack of a denicotinized cigarette or sham smoking (i.e., puffing on an unlit cigarette) condition was one limitation of this study (Ray et al., 1986). The somatosensory effects of smoking along may have had effects on blood pressure and other outcome measures.

Another clinical evaluation that examined the individual and combined effects of nicotine and caffeine recruited 10 participants who were non-smokers and regular caffeine users (Smits et al, 1993). All participants completed four sessions that differed by drug administered: combination of nicotine (4 mg gum) and caffeine (250 mg i.v.), placebo gum and caffeine (250 mg i.v.), nicotine (4 mg gum) and placebo infusion, and placebo gum and placebo infusion. Outcomes measures included blood pressure, which was increased during the nicotine, caffeine, and the combination conditions. To determine whether the combination of nicotine and caffeine differed from the effects of each drug administered individually, the individual effects were summed and compared to the combination (i.e., effect-addition model; Wessinger, 1986). Although there was slight trend of the combination to produce higher blood pressure than the sum of the nicotine's and caffeine's effects, there was no significant difference between the two. The researchers here deemed that the combination of nicotine and caffeine produced an "additive" (i.e., effect-additive) effect upon blood pressure, larger than either drug administered individually but not significantly different when compared to these individual effects added together (Smits et al., 1993). Importantly, these researchers recognized that the route of

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administration may have affected their results. Nicotine gum delivers nicotine at a much slower rate than cigarette smoking and the lower plasma nicotine levels achieved during this study may have affected the interaction between nicotine and caffeine (Smits et al., 1993).

Similar to preclinical work, enhancement of the discriminative and subjective effects of nicotine by acute caffeine doses has been examined in the clinical laboratory. Twenty smokers who used caffeine regularly were trained to discriminate between placebo and 1 mg nicotine chewing gum (Duka, Tasker, Russell, & Stephens, 1998). Generalization was then tested (0, 0.25, 0.5, 1 mg nicotine) with either a placebo or 50 mg caffeine preload (via capsule). The caffeine preload increased subjective ratings of "stimulated" and "alert" at the 0 mg nicotine dose, and "jittery" at the 0.5 and 1 mg nicotine dose. In addition, the caffeine preload partially substituted for nicotine at the 0 mg nicotine dose. While this study provided little support for a caffeine-induced enhancement for the subjective qualities of nicotine, caffeine's partial substitution demonstrated the possibility of common interoceptive stimulus properties of nicotine and caffeine (Duka et al., 1998).

The effects of chronic caffeine administration upon nicotine-related subjective effects have also been assessed in smokers within the clinical laboratory. One long-term study compared the effects of nicotine (0, 1.0, and 2.0 mg/70 kg i.v.) observed in cigarette smokers maintained on caffeine as compared to when they were caffeine abstinent in a within-subjects, double-blind, clinical laboratory study (Jones & Griffiths, 2003). Outcome measures included subjective ratings of VAS items "Do you feel a rush?", "Do you feel a drug effect?", and "Do you feel stimulated?". Relative to ratings observed during caffeine abstinent condition,

participant ratings of "drug effect" and "stimulated" were significantly higher after the 2.0 mg/70 kg nicotine dose during caffeine maintenance (e.g., peak change "stimulated" scores for caffeine maintenance group = approximately 30 points and for the caffeine abstinence group = approximately 17 points). These results are consistent with the caffeine maintenance adding to the effect of the 2.0 mg/70 kg i.v. nicotine dose, but without a caffeine maintenance group exposed to placebo infusions or a placebo control group, the study design lacks the ability to allow the authors to make this inference (Jones & Griffiths, 2003).

Additional evidence supports the potential for an effect-additive effect of nicotine and caffeine on measures of cardiovascular and subjective effect. Nineteen smokers who regularly drank coffee were assessed using a within-participants, placebo control design in which they received nicotine (15 μg/kg, intranasally) and caffeine (5 mg/kg, p.o.) in combination, and the effects were compared to these doses of nicotine alone, caffeine alone, and placebo during both rest and activity periods (Perkins et al., 1994). Outcome measures included tension and arousal scales of POMS, VAS items (e.g., "jittery", "dizzy"), and cardiovascular effects (heart rate and blood pressure). The POMS scale of arousal revealed a main effect of caffeine and nicotine, and during the rest period, mean change from baseline scores indicated the summed individual drug effects versus the combination of nicotine and caffeine were not significantly different (i.e., effect-additive; Wessinger, 1986). Interestingly, the individual and combination effects of nicotine and caffeine on arousal were attenuated during the activity period. The POMS scale of tension revealed a significant interaction between caffeine and nicotine and the mean change from baseline score during the combination was observed to be greater than the sum of the

individual drug effect scores (i.e., greater than effect-additive) during both the activity and rest periods. Similarly to the POMS scale of tension, for the VAS item "jittery" mean change from baseline scores were observed to be greater during the combination than the sum of the individual drug effect scores, but only during the rest period. During the activity period, changes were only effect-additive (i.e., approximately equal between the combination and the sum of individual drug effects). In contrast for the VAS item "dizzy", nicotine increased scores during both activity and rest, while caffeine only increased reports during rest. For cardiovascular reports, nicotine increased heart rate and blood pressure and caffeine decreased heart rate and increased blood pressure during both activity and rest. Similar to previous observations, during the combination of nicotine and caffeine effect-additive effects were observed for blood pressure. In summary, this study showed evidence of an enhancement of subjective effects (e.g., arousal, tension, 'jittery", and "dizzy") under some conditions and enhanced blood pressure effects during nicotine and caffeine co-administration and is one of the few examinations that provides evidence for greater than effect-additive effects concerning the co-administration of these two drugs (Perkins et al., 1994).

Another within-subject clinical laboratory study has shown both the attenuation and enhancement of nicotine-related effects by caffeine (Rose & Behm, 1991). Twelve smokers who regularly drank coffee received nicotine (approximately 0.75 mg, via cigarette) and caffeine (150 mg, p.o.) in combination, and the effects were compared to these doses of nicotine alone, caffeine alone, and placebo. Measures included the arousal and tension scales from the POMS and blood pressure. Results indicated that caffeine attenuated the nicotine induced increase in

participant-rated arousal when compared to the increases observed during the nicotine only condition. During the caffeine only and caffeine in combination with nicotine condition, relative to the no caffeine conditions there were significant increases in mean diastolic blood pressure. The combination of drugs produced the highest increase in mean diastolic blood pressure, which appeared higher than the sum of nicotine alone and caffeine alone. Unfortunately, the researchers did not examine these results in terms of the effect-addition model of interaction (Rose & Behm, 1991). Common to other studies of caffeine and nicotine co-administration, sample size could be a limiting factor of these results. Interestingly, the attenuation effect of caffeine upon nicotine's subjective effects under resting conditions observed in this study (Rose & Behm, 1991) is inconsistent with some findings (Perkins et al., 1994, observed attenuation only under activity conditions), but the enhanced increase of blood pressure during the nicotine/caffeine condition is consistent with other results (Smits et al., 1993; Ray et al., 1986; Perkins et al., 1994).

Other clinical laboratory research provides less support for the notion that caffeine enhances nicotine's effects, at least on some outcome measures (Perkins et al., 2005; Blank et al., 2007; Kerr, Sherwood, & Hindmarch, 1991; Pritchard, Robinson, deBethizy, Davis, & Stiles, 1995). A double blind within-subject study of five non-smokers and five smokers measured performance on a variety of cognitive measures (Kerr et al., 1991). Participants were administered caffeine (300 mg p.o.) and nicotine (2 mg gum), caffeine placebo and nicotine (2 mg gum), caffeine (300 mg p.o.) and nicotine placebo, and caffeine placebo and nicotine placebo. The results indicated that the combination of nicotine and caffeine produced no greater

effects on the cognitive tasks relative to each drug administered individually. The relatively small sample size and lack of control for the varied nicotine history of the participants may limit the applicability of these findings (Kerr et al., 1991). Another study that did not find evidence for enhancement between nicotine and caffeine recruited only non-smoking moderate caffeine users (Blank et al., 2007). This double blind, within-subject examination consisted of four sessions where twenty participants were preloaded with 0, 75, or 150 mg caffeine via capsule prior to each nicotine administration (2 and 4 mg). Measures including physiological and subjective ratings were taken periodically. Nicotine increased heart rate and blood pressure significantly, but these increases were independent of caffeine dose administered. In addition, no significant main effects of caffeine were observed for any of the subjective items. This study provided little evidence that the effects of nicotine are influenced by concurrent caffeine administration. Limitations for this study included a small sample size and lack of a nicotine placebo (Blank et al., 2007).

In sum, clinical laboratory studies provide some evidence of common interoceptive stimulus properties of nicotine and caffeine and effect-additive effects on some subjective measures (e.g., arousal, "stimulated"," jittery", and "dizzy") and blood pressure. Taken with the preclinical work demonstrating a potential interaction during nicotine and caffeine co-administration, these two drugs when administered in combination may induce heightened reward/reinforcement-related effects that could impact patterns of tobacco use initiation and maintenance. In addition, examination of these caffeine co-administration studies highlights the need for controlled study design, careful attention to sample size, and use of outcome measures

sensitive to the combined effects of nicotine and caffeine. Importantly, a study examining the

combined effects of nicotine and caffeine should include conditions where each drug is presented

alone and in combination (as in Perkins et al., 1994; Kerr et al., 1991; Smits et al., 1993; Rose &

Behm, 1991) as this design allows for systematic comparison and determination of the effects of

combination, as well as the use of the effect-addition model of interaction.

Novel Caffeine and Nicotine Co-Administration: Caffeinated Tobacco

In the past, nicotine and caffeine co-administration outside the clinical laboratory has

been limited by the products available (see Table 2). Generally, users achieve co-administration

in the form of caffeinated beverages or pills used in the combination with tobacco or nicotine

replacement products. With the advent of caffeinated tobacco products, the potential for

simultaneous, single product administration of nicotine and caffeine exists. Most recently,

caffeinated smokeless products (Revved Up Energy Dip, Elixyr Power Energy Snus, and

Northern Energy Snus) have been marketed to users as a "substitute/complement for energy

drinks and coffee" (Elixyr Power Energy Snus; buysnus.com) and "a convenient and discreet

way to enjoy smokeless tobacco and achieve increased energy levels" (Revved up Energy Dip;

southernsmokeless.com). In addition, a brand of caffeinated waterpipe tobacco (Tangiers F-

Line) has been available for the past five years. While these products have not received

systematic study, anecdotal reports suggest the possibility of enhanced effects. For example

regarding Tangiers F-line caffeinated waterpipe tobacco, users write "Tangiers caffeinated line

of hookah tobacco will knock you out of your boots" (TimL, 9/19/08, Yelp.com), and "If regular

Tangiers can give you a massive buzz, the F-Line brings it to a whole new level!" (Zeodynamic,

Table 2.

Caffeine content for a variety of consumer products

Product	Mean caffeine (mg)
Coffee	
Generic filtered (8 oz) [1]	133
Generic instant (8 oz)[1]	66
Tea (depending on brew) [1]	53
Food	
Haagen-Dazs Coffee Ice Cream (8 oz)[1]	10
Hershey's Chocolate Bar (1.45 oz)[1]	36
Soft drink	
Coca-Cola Classic (12 oz) [1]	35
Mountain Dew (12 oz) [1]	71
Energy drink	
AMP (8.4 oz)[1]	74
Red Bull (8.3 oz)[1]	80
Over-the-counter drugs	
No Doz (1 tablet)[1]	200
Vivarin (1 tablet)[1]	200
Anacin (2 tablets)[1]	64
Excedrin (2 tablets)[1]	130
Tobacco Products	
Elixyr Power Energy Snus (per portion)[2]	28 mg
Northern Energy Snus (per portion)[2]	20 mg
Revved Up Energy Dip	N/A[3]
Tangiers F-Line	N/A[3]

[1] Center for Science in the Public Interest, 2007
[2] Data available at http://thenortherner.com.
[3] No data available from manufacturer.

6/28/07, Hookahpro.com). These anecdotal reports suggest that when caffeinated tobacco

products are heated and the smoke inhaled the resulting effect may be greater than those

experienced when inhaling the smoke of non-caffeinated products. Of course the validity of this

notion rests on the idea that volatilized caffeine is physiologically active.

Volatilized Nicotine and Caffeine

Nicotine delivered by inhalation of volatilized tobacco smoke particles (e.g., smoking)

has been examined in a large number of empirical studies (e.g., Hoffman & Hoffman, 1997).

Because inhaled drugs escape first-pass intestinal and hepatic metabolism, they are rapidly

absorbed and enter of the brain more quickly than those administered via other methods

(Benowitz, 2008). Many believe the speed of entry of nicotine into the brain by smoking is a

strong determinant of the reinforcing effects and abuse liability of a drug (Farré & Cami, 1991;

Benowitz, 2008). A number of other drugs of abuse are consumed by the inhalation route

including marijuana, cocaine, and opiates, perhaps because access to the brain is so rapid with

this method. Little research has been performed concerning the delivery characteristics of

volatilized caffeine, but if, like other drugs of abuse, this method of administration produces

more rapid access to the brain, it is not unlikely that inhaled caffeine may offer a more

heightened abuse liability than other modes of ingestion.

Three studies have analyzed samples of caffeine post-volatilization (Klous, Lee,

Hillebrand, et al., 2006; Klous, Lee, Van den Brink, et al., 2006; Brenneisen & Hasler, 2002) to

determine delivery characteristics, and one clinical laboratory study has examined the

pharmacokinetics of volatilized caffeine in humans (Zandvliet et al., 2005). Importantly all of

these studies have utilized the anhydrous base form of caffeine (i.e., 1,3,7-Trimethylxanthine, $C_8H_{10}N_4O_2$) not the salt form (i.e., caffeine citrate; $C_{14}H_{18}N_4O_9$). One examination demonstrated that caffeine was a heat stable compound after preparations underwent temperatures of 250-400 C° with 0% of the caffeine compound experiencing degradation (Brenneisen & Hasler, 2002).

Other studies produced similar results with near complete recovery of caffeine from mixtures that were heated by standardized methods to 200-350 C° (Klous, Lee, Van den Brink, et al., 2006) or by samples heated by human participants to similar temperatures (Klous, Lee, Hillebrand, et al., 2006). In the only clinical study of volatilized caffeine, 10 volunteers inhaled the smoke produced by heating a preparation with a lighter or a heating plate containing 100 mg caffeine (anhydrous base) on five separate sessions (Zandvliet et al., 2005). During each session blood was sampled at baseline and at standardized intervals after inhalation (inhalation period averaged 10 minutes; 1, 2, 5, 7.5, 10, 15, 22.5, 30, 45, 60, 120, 240, and 480 minutes). Plasma was analyzed from each sample for caffeine levels and four caffeine metabolites. Participants' plasma samples that indicated recent caffeine ingestion prior to session were excluded from the analyses. According to the researchers' pharmacokinetic modeling, caffeine appeared in circulation rapidly following inhalation; this finding indicated absorption of caffeine from the lungs (Zandvliet et al., 2005). Estimates also indicated that inhalation of 100 mg caffeine produced comparable plasma caffeine concentrations as drinking a beverage with 80 mg of caffeine. One limitation of these results was the lack of plasma concentration data available during inhalation, thus the speed of absorption was not accessed. As speed of absorption is an important factor in the abuse ability of a drug and inhalation has been shown to be a rapid

method of absorption and distribution, the absence of this outcome is disappointing. Fortunately, the elimination rate of caffeine corresponded well with previous reports of p.o. and i.v. caffeine administration (Bonati et al., 1982; Renner, Wietholtz, Huguenin, Arnaud, & Preisig, 1984) demonstrating that the inhaled caffeine metabolizes at a similar rate compared to other administration routes (Zandvliet et al., 2005). In sum, these studies demonstrate that caffeine can be volatilized without degradation to its chemical properties and volatilized caffeine can be inhaled and metabolized similarly to other routes of administration (Brenneisen & Hasler, 2002; Zandvliet et al., 2005).

These results along with the anecdotal reports described earlier support the idea that smoking caffeinated tobacco may expose users to active caffeine doses. The extent to which those caffeine doses interact with tobacco-delivered nicotine is another question.

Evaluating the Effects of Caffeinated Waterpipe Tobacco

Evaluations examining the interaction of nicotine and caffeine described in the preceding sections demonstrate many techniques and methods than can be applied to examine the effects of caffeinated waterpipe tobacco in the clinical laboratory. While other study designs are used, within-subject methods are most common (e.g., Blank et al., 2007; Rose & Behm, 1991; Smits et al., 1993; Perkins et al., 1994). Outcome measures also differ across studies, though plasma nicotine, cardiovascular response, and detailed subjective effect measures are particularly common in assessing effects and interactions of these two drugs. Study duration is also an important variable. Short term examinations can be more feasible and practical (see Perkins et al., 1994), while longer term studies may better replicate actual dosing patterns in humans (e.g.,

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Emurian et al., 1982; Jones & Griffiths, 2003). Longer term studies may be also be more difficult and expensive to perform. Blood samples collected before and after drug administration address the exposure associated with acute administration of nicotine and caffeine (e.g., Shafagoj et al., 2002; Zandliviet et al., 2005). Similarly, subjective questionnaires administered before and after provide valuable information concerning pleasurable and adverse subjective effects and direct effects (e.g., dizzy, nausea, and lightheadedness) associated with nicotine and caffeine administration. Designing a study that examines these two drugs must take into consideration peak effects and duration of action. As caffeine's half-life is longer and duration of peak effects occurs over much longer time than nicotine, the time course of subjective and physiological assessment should reflect these pharmacokinetic differences. Appropriate controls are another important study design consideration. Specifically, designs that examine an interaction should include conditions where each factor of interest is examined individually and in combination, as well as a placebo control. These measures ensure effects can be examined discretely (see Perkins et al., 1994; Rose & Behm, 1991) as well as provide the ability to utilize pharmacological models of interaction such as the effect-addition model (Wessinger, 1986). Lastly, previous studies examining the interaction of caffeine and nicotine co-administration may have used inadequate sample sizes (see Blank et al., 2007), so that an increased sample size to examine this pharmacological interaction may be warranted. Thus, clinical laboratory studies that use acute exposure methodology can address many questions related to effects of nicotine and caffeine use individually and in combination.

Statement of the Problem

Caffeine and nicotine are the two of most commonly consumed licit psychoactive drugs in the world. Nicotine is usually administered via tobacco products and caffeine is most often consumed orally via beverages. Tobacco use is associated with nicotine dependence and is considered the leading cause of preventable death in the U.S., while caffeine is considered a drug with low abuse liability and little harm. The co-administration of caffeine and nicotine-containing products is common: over 86% of cigarette smokers report using caffeine versus 77% of non-smokers, and this observation may reflect an interaction between these two drugs. Some research suggests the combination of nicotine and caffeine may produce effects that are more rewarding or pleasurable than either drug alone. This reward enhancement may also influence patterns of tobacco use initiation and maintenance. Alarmingly, despite decreases in cigarette smoking prevalence over the past twenty years, an alternative tobacco use method, waterpipe tobacco smoking, is experiencing recent popularity in the U.S. This method of tobacco smoking, especially popular among young adults, may allow for increased opportunities for nicotine and caffeine co-administration via a caffeinated tobacco product (Tangiers F-Line). This product enables users, who may be among an age group that is especially vulnerable to tobacco use initiation, to consume this combination of drugs in a single form. Data concerning "smoked" (i.e., volatilized) caffeine are sparse, but reports indicate it is absorbed and metabolized similarly to other methods of caffeine administration. Taken with the evidence from previous nicotine and caffeine co-administration studies, this caffeinated tobacco product may produce enhanced reward-related effects in users compared to typical waterpipe tobacco preparations. An

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empirical laboratory study that assesses both the effects of caffeinated waterpipe tobacco as well as describes the effects of volatilized caffeine is both a feasible and practical option.

The Present Study

The aims of this study were to compare, using a within-subject, factorial design, the subjective and cardiovascular effects of smoking caffeinated waterpipe tobacco with the effects of smoking waterpipe preparations containing nicotine and no caffeine, low nicotine and caffeine (a preparation with no nicotine and caffeine was desired but was unavailable), or neither nicotine nor caffeine. This study design allowed us to examine the effects of the combination of nicotine and caffeine in comparison to low nicotine and caffeine, nicotine alone, and a placebo. This experiment also was the first systematic evaluation of the physiological and subjective effects associated with the volatilized caffeine.

Statement of Hypothesis

The primary goal of the study was to examine caffeinated waterpipe tobacco in comparison to non-caffeinated tobacco, and the primary hypothesis reflects this goal: caffeinated waterpipe tobacco would produce some cardiovascular and subjective effects that are greater than non-caffeinated waterpipe tobacco.

Secondary hypotheses include: caffeinated and non-caffeinated waterpipe tobacco preparations would increase plasma nicotine levels while the placebo preparation would not, the caffeinated waterpipe tobacco and low nicotine caffeinated preparations would increase plasma caffeine levels, and the nicotine-containing preparations would induce subjective effects characteristic of acute nicotine administration.

Method

Selection of Participants

Thirty-two waterpipe tobacco smoking community volunteers (16 men) completed this within-subject, Latin-square ordered study. Power analysis suggested that thirty participants were necessary to detect a moderate effect size (i.e., $f \geq 0.35$) with a small or moderate correlation between repeated measures (i.e., $r \geq 0.50$) with a power of 0.80 and alpha level < 0.05 (Barcikowski & Robey, 1985). While forty participants were proposed initially to maximize study sensitivity to detect an interaction, based upon the preliminary analysis of the first twenty participants, the lack of caffeine exposure detected supported the use of fewer participants and early closure of the study. To continue to enroll participants in a study that cannot address its hypotheses presents an unacceptable risk/benefit ratio (see Chapter 3 of the Institutional Review Board Guidebook, 1993). The thirty-two participants ensured that 8, 4-condition Latin squares could be completed to minimize order effects.

Inclusion criteria. Participants were included if they reported that they were healthy, between 18 and 50 years of age, reported smoking tobacco using a waterpipe at least four times a month for the past 6 months (ensured participants were not exposed to waterpipe smoke toxicants above what they usually consume), and reported daily caffeine use of at least 100 mg (e.g., 1 cup of caffeinated coffee) for the past year (see Jones & Griffiths, 2003; Perkins et al., 1994). All participants provided informed consent and agreed to abstain from all tobacco/nicotine-containing products and caffeine/caffeinated beverages for at least 12 hours prior to each of four required sessions.

Exclusion criteria. Individuals were excluded if they reported a history of chronic health problems or psychiatric conditions. Women were also excluded if they tested positive for pregnancy (assessed by urinalysis during screening) or reported current breastfeeding. Regular use of cigarettes (> 5 cigarettes/day) for the past year was exclusionary (participants with a higher cigarette use history may have been nicotine dependent and had more difficulty abstaining from tobacco use). In addition, regular use of prescription medication (except for vitamins or birth control) was also exclusionary to reduce any potential drug interaction with nicotine and caffeine administration (Zevin & Benowitz, 1999). Any potential participant reporting a current attempt to cease tobacco use was excluded and referred to a smoking cessation treatment provider. Participants were excluded if they reported past month use of opioids or cocaine or had a positive urine drug screen for these drugs; individuals who reported > 5 days of marijuana use in the past month or > 20 days of alcohol use in the past month were also excluded. To reduce flavor bias for the products used in the current study, participants who reported "Melon" as their favorite waterpipe tobacco flavor were excluded (see *Materials*).

Screening and Informed Consent Procedures

All individuals interested in participating in the study completed a two-part screening process. The first part consisted of a phone interview where potential participants responded to questions about their health and tobacco use (see Appendix A). Individuals whose responses suggested that they were eligible were invited to appear in the laboratory for an in-person screening. Prior to completing the in-person screening, individuals provided their informed consent to participate in the study (see Appendix B). They then provided information

concerning their health, tobacco use, and basic demographics. In addition, a urine sample was required for immediate semi-quantitative analysis of illicit drug use and, for women, a pregnancy test.

A total of 48 participants (22 women) consented to participate in the study. However, 15 failed the in-person screening process (i.e., 8 did not report consuming at least 100 mg caffeine daily for the past year; 7 did not meet other inclusion criteria). In addition, one participant was discontinued after the first two sessions due to high blood pressure. Of the 32 participants who provided complete blood plasma, cardiovascular, subjective and topography results, 16 were men (10 non-white) and 16 were women (12 non-white). These participants were on average, 21.6 years old (SD = 2.7) and reported smoking tobacco using a waterpipe 11.4 occasions/month for the past 2.2 years (SD = 1.6). Eleven of the participants smoked cigarettes (on average 2 cigarettes/day for 23.5 months). These participants also reported consuming on average 308.1 mg (SD = 181.6) of caffeine per day for the past 4.5 years (SD = 3.1).

Materials

Conditions differed by waterpipe preparation: combination of caffeine and nicotine (CAFF/NIC; Tangiers F-Line), caffeine and reduced (low) nicotine (CAFF/LN; low nicotine Tangiers F-Line), nicotine and no caffeine (NIC; Tangiers original), or neither caffeine nor nicotine (PLACEBO; Soex; a sugarcane based waterpipe product, see www.soex.com/herbalmolasses.html). Table 3 displays the nicotine and caffeine content for each product. The melon flavor was the only flavor available in all four varieties and was the flavor

Table 3.

Mean (standard deviation) nicotine and caffeine content analysis of study materials.[a]

	Condition			
	PLACEBO	NIC	CAFF/LN	CAFF/NIC
Nicotine (mg/g)	<LOQ[b]	3.25 (0.07)	0.846 (0.005)	3.82 (0.08)
Caffeine (mg/g)	Not tested	Not tested	0.481 (0.012)	0.507 (0.026)

[a] Arista Laboratories ISO 17025 accredited.
[b] Lower than limit of quantification (LOQ).

used for all sessions. In order to control for flavor preference, we excluded all individuals who reported that "Melon" was their preferred flavor. Thus, for all study participants, the flavor used in this study was a non-preferred one (a similar technique has been used to reduce cigarette brand bias, see Buchhalter, Schrinel, & Eissenberg, 2001).

Procedure

After all screening procedures were completed (including informed consent), the first of four experimental sessions was scheduled, with each session corresponding to one of the four product conditions (i.e., CAFF/NIC, CAFF/LN, NIC, or PLACEBO). The four double-blind, Latin-square ordered sessions occurred at least 48 hours apart, and lasted for approximately 3 hours. On each session day, participants reported to VCU's Clinical Behavioral Pharmacology Laboratory at a pre-determined time (time of day varied between subjects, but was constant within-subject) and their expired air CO was assessed as a measure of compliance with theovernight tobacco abstinence criterion (CO $\leq$ 10 parts per million [ppm] is an indicator of overnight abstinence; see Breland et al., 2002; Buchhalter et al., 2001; Schuh, Schuh, Henningfield, & Stitzer, 1997). If the initial CO assessment did not indicate abstinence, participants could wait until their CO level reached criterion, or they could reschedule the session for another day. In addition, participants were asked to provide a saliva sample for "verification" of greater than 12 hours of caffeine abstinence, though the sample was not tested (bogus pipeline method used to improve participant compliance; see Rose & Behm, 1991; Nastase, Ioan, Braga, Zagrean, & Moldovan, 2007). Overnight tobacco and caffeine abstinence

avoids the effects of acute tolerance to nicotine's and/or caffeine's cardiovascular effects (Perkins et al., 1995; Whitsett, Manion, & Christensen, 1984).

Once the participant met the pre-session CO criterion and provided a saliva sample, a catheter was placed in a forearm vein and the session began with continuous measurement of physiological response during a 20-minute adaptation period followed by a 10-minute baseline period. Once the baseline period was complete, a baseline expired air CO was measured, participants responded to subjective measures, and 15 ml of blood was sampled. Participants then began a 45-minute, double-blind waterpipe use period.

In this study, laboratory staff with no participant contact packed the waterpipe head with 10 g (as in Shihadeh, 2003) of the day's preparation (i.e., CAFF/NIC, CAFF/LN, NIC, or PLACEBO). For smoking, we adopted a variant of the protocol used elsewhere (Shihadeh, 2003): the loaded head was covered with a 9 cm x 9 cm piece of aluminum foil that was perforated using a "screen pincher" that standardizes the number and size of the holes in the foil (see www.smoking-hookah.com). Initially, a single quick-light charcoal disk (Three Kings, Holland; this brand is used in Richmond waterpipe cafés) was lit and placed on the foil. Laboratory experimentation reveals that a single disk is insufficient for an average use episode (it yields smoke-free puffs at the end), and naturalistic observation makes clear that waterpipe users exhibit idiosyncratic behavior regarding charcoal application. We made available several (pre-weighed) ½ charcoal disks that participants could add to the top of the head, *ad libitum*. Also for standardization, the waterpipes used in all studies were of the same brand/size used in the waterpipe cafés found in the area surrounding Richmond VA (MYA, 23 inch), with 870 ml of

water placed into the base. In every session, the waterpipe hose was tipped with a new, sterile, disposable mouthpiece.

Participants smoked *ad libitum,* and puff topography was measured throughout the 45-minute smoking session. At baseline and 5, 15, 30, 45, 60, 90, and 120 minutes after use began, 15 ml blood was sampled and subjective measures were administered. During each of the four sessions, participants could watch a movie of their choice on a laboratory-provided DVD player, read, or listen to music; importantly, these activities were restricted during completion of any of the subjective questionnaires. The session terminated 5 minutes after the last subjective assessment was completed, the catheter removed, and if necessary, another session was scheduled. The total amount of blood taken in a single session was 120 ml and for the entire 4-session study was 480 ml (slightly more than the ~450 ml taken in a single blood donation). Participants who completed the study were paid $350.

Physiological Measures

Plasma nicotine and caffeine. Plasma nicotine is relevant to waterpipe tobacco smoking's acute effects and is often measured in studies examining the short-term effects of tobacco products in tobacco smokers (e.g., Breland et al., 2002). Plasma caffeine levels were used to indicate how much volatilized caffeine is being delivered to the smoker. After 15 ml blood collected was collected at each sampling time point, the sample was centrifuged and two separate aliquots of plasma were frozen immediately at $70^{\circ}C$ for analysis of nicotine and caffeine concentrations. For plasma nicotine, standard methods (described in Breland et al., 2006) include a limit of quantification (LOQ) of 2 ng/mL (as in Gray, Breland, Weaver, & Eissenberg,

2008; Cobb, Weaver, & Eissenberg, 2010). Caffeine in plasma were analyzed by reverse phase chromatographic separation (Shimadzu pump systems, Phenomenex Gemini column [C18, 110Å, 100mm X 2mm, 5µm column]) in hyphenation with the tandem mass spectrometric determination (Waters Quattro API Micro, Triple Quadrupole Instrument with Masslynx 4.1 software). The assay method was linear from 20 to 20000 ng/mL and results were quantified by linear regression method with $1/X^2$ weighting (see Figure 3 for the calibration/standard curve).

Cardiovascular effects. Heart rate was monitored every 20 seconds while systolic and diastolic blood pressure were measured every 5 minutes (Noninvasive Patient Monitor model 507E, Criticare Systems, Waukesha, WI).

Expired air carbon monoxide (CO): Expired air CO was recorded by the research assistant at 50, 60, 90, and 120 minutes post product administration (BreathCO monitor, Vitalograph, Lenaxa, KS).

Subjective Measures

During each session, participants responded to computerized questionnaires (each questionnaire administered a total of 8 times per session). These questionnaires consisted of the Direct Effects Scale, the Profile of Mood States, and the Positive and Negative Affect Scale.

The Direct Effects Scale (DES)*:* This measure was developed using reports describing the effects of nicotine and caffeine administered individually and in combination (Perkins et al., 1994; Jones & Griffiths, 2003; Blank et al., 2007; Garrett & Griffiths, 2001; Pullan et al., 1994; Gourlay, Forbes, Marriner, Pethica, & McNeil et al., 1995; Liguori, Hughes, & Grass, 1997).
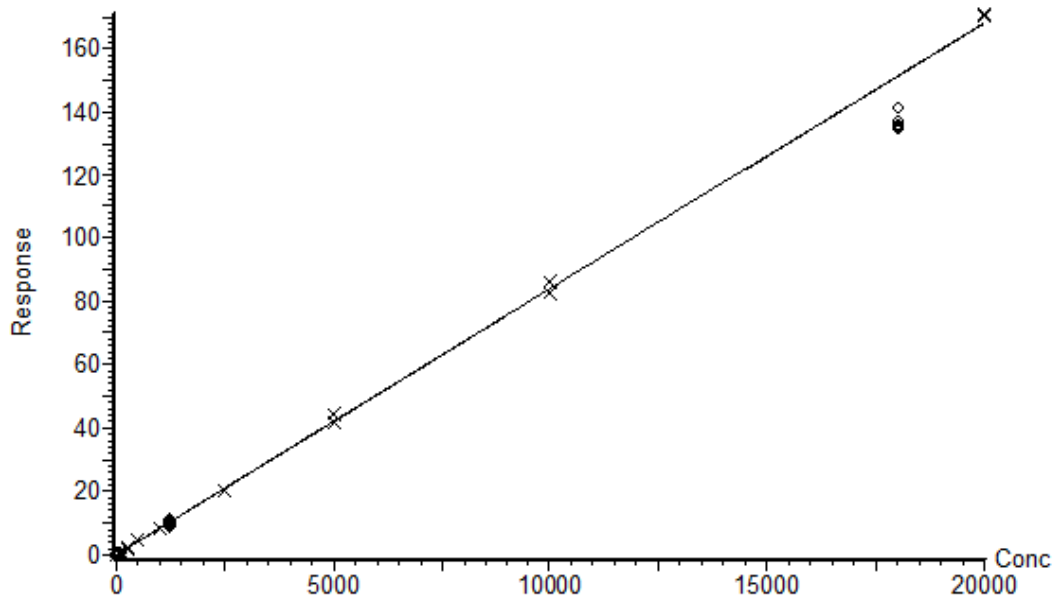
Figure 3. Caffeine assay calibration/standard curve. X symbols denote standards and diamonds

represent quality controls tested. Concentration on the X-axis is caffeine concentration in ng/ml,

and response on the Y-axis is the peak area of caffeine / peak area of caffeine d-3. Peak area is

obtained from the individual chromatogram of each sample or standard when caffeine is the

analyte and caffeine-d3 is the internal standard.

These computerized items were presented as a visual analog scale (VAS) consisting of a word or phrase above a horizontal line anchored on the left with "Not at all" and on the right with "Extremely". Participants used the mouse to make a mark along the horizontal line and item scores were calculated as a percentage responding the mark's distance from the left anchor. The 18 VAS (0-100) items are: "Nauseous", "Dizzy", "Lightheaded", "Nervous", "Sweaty", "Headache", "Excessive salivation", "Heart pounding", "Confused", "Weak", "Hungry", "Do you feel a rush?", "How high are you?", "Do you feel any drug effects?", "Do you like the drug effects?", "Do you dislike the drug effects?", "Do you feel any good drug effects?", and "Do you feel any bad drug effects?".

The Profile of Mood States (POMS): The POMS (McNair et al., 1971) consists of 65 items relating to mood that are rated on a 5-point scale ranging from 0 (Not at all) to 4 (Extremely). Items were reported as six previously defined factors: anger/hostility, confusion/bewilderment, depression/dejection, fatigue/inertia, tension/anxiety, and vigor/activity. Common to a few examinations of the concurrent effects of nicotine and caffeine (Perkins et al., 1994), the POMS composite scale of arousal was assessed by adding the tension/anxiety and vigor/activity scales and subtracting the fatigue/inertia and confusion/bewilderment scales (de Wit, Pierri, & Johanson, 1989). The POMS measure has been used extensively in studies examining the effects of concurrent nicotine and caffeine administration (Chait & Griffiths, 1983; Rose & Behm, 1991; Oliveto et al., 1991; Perkins et al., 1994).

The Positive and Negative Affect Scale (PANAS): The PANAS (Watson et al., 1988) consists of 20 items: "Enthusiastic", "Inspired", "Attentive", "Active", "Alert", "Excited",

"Determined", "Strong", "Proud", "Interested", "Upset", "Hostile", "Ashamed", "Distressed", "Guilty", "Irritable", "Afraid", "Scared", "Nervous", and "Jittery". Participants rated each item on a 5-point scale ranging from 0 (Not at All) to 4 (Extremely). Items were then collapsed into two factors previously defined by factor analysis: positive affect and negative affect. Positive affect reflects the extent to which a person feels enthusiastic, active, and alert; high positive affect is characterized by high energy, full concentration, and pleasurable activity (Watson et al., 1988). Negative affect generally indicates distress and displeasure; low negative affect implies a state of calm and peacefulness (Watson et al., 1988). This measure has been used in previous studies of nicotine's interactions with other concurrently administered drugs (Sofuoglu, Waters, & Mooney, 2008; Epstein & King, 2004) and examinations of concurrent nicotine and caffeine administration (Blank et al., 2007).

Puff Topography

Puff topography is a sensitive measure of drug self-administration in cigarette smokers that has been used for decades (e.g., Robinson & Forbes, 1975; Herning et al, 1981; Brauer et al., 1996). Waterpipe puff topography was measured with a non-invasive device developed for this purpose (Shihadeh, Antonios, & Azar, 2005). In order to assess waterpipe topography, a pressure transducer was integrated into the waterpipe hose, and inhalation-induced pressure changes are amplified, digitized, and sampled. Software converts signals to air flow (ml/sec) and integrates the flow data, producing measures of average puff volume, average puff duration, total smoke volume, number of puffs, and average interpuff interval. Digital records of puff topography were made for all sessions.

Data Analysis Plan

All data analyses were performed using IBM SPSS (version 19). Plasma nicotine results below the LOQ were replaced with the LOQ (i.e., 2 ng/ml; Gray et al., 2008; Cobb et al., 2010), and plasma caffeine results below the LOQ were replaced with the LOQ (i.e., 20 ng/ml). Heart rate data was analyzed by averaging values in five-minute bins for the duration of the study as in previous work (Blank et al., 2007; Cobb et al., 2010). Across all subjective, topography, and physiological measures (except for systolic and diastolic BP) less than 0.4% of data were missing. For these outcomes, the average of the value before and after was used to impute missing data. For systolic and diastolic blood pressure 6% of data were missing, and multiple imputation methods (MI) were employed via IBM SPSS (Rubin, 1987; Schafer, 1997; Little and Rubin, 1987). The MI procedure generates imputed datasets by estimating missing values using a Markov chain Monte Carlo technique. Imputed datasets (at least 5) are then analyzed separately using user-specified statistical analyses, and results are combined using set rules (Rubin, 1987) or by averaging the analysis results of each imputed dataset. Ten imputed datasets were generated for systolic and diastolic BP and results (see time course and peak change) are based on averaging the analysis results for each of these imputations.

The primary outcome measures (physiological, puff topography, and subjective effects) were analyzed in two ways. First, to characterize the time course of effects, data were analyzed using a three-factor repeated measures analysis of variance (ANOVA) where the factors are nicotine (yes/no), caffeine (yes/no) and time (baseline, 5, 15, 30, 45, 60, 90, and 120 for plasma and subjective effect data). Second, in order to address specifically the effects of nicotine and

caffeine in combination, time course data in each of the four conditions was converted to peak change scores (i.e., baseline value subtracted from all subsequent values and then the highest value observed is the peak; Childs et al., 2008). Peak change scores were used initially to examine any effects associated with condition order (i.e., the order of sessions for each participant). For these peak change repeated measures ANOVA analyses, condition order (1, 2, 3, 4) was used as a between subjects factor and the two within-subjects factors were nicotine (yes/no) and caffeine (yes/no). Of the 198 main effects and interactions analyzed, 10 were expected by chance and only 8 interactions involving the between subjects factor were significant ($p < 0.05$). Because these significant results may reflect chance rather than a real difference between the effects of different condition orders, the between-subjects factors was dropped and all analyses were repeated using the within-subjects factors only. In addition, puff topography data were analyzed by a two-factor repeated measures ANOVA where the factors were nicotine (yes/no) and caffeine (yes/no). For all analyses, Huynh-Feldt corrections was used to adjust for violations of sphericity (Huynh & Feldt, 1976) and Tukey's Honestly Significant Difference (Keppel, 1991) was used to explore differences between means (as in Breland et al., 2006; Gray et al., 2008; Cobb et al., 2010).

Results

Time Course Analyses

Table 4 displays time course statistical analyses (main effects and interactions) for all measures. Interactions that involve the time factor and either the nicotine and/or caffeine factors are the most relevant as they indicate that the results observed differed across time and between nicotine and caffeine-containing products.

Physiological measures. As Table 4 shows, a significant nicotine by time interaction ($p<0.05$) was observed for plasma caffeine. As displayed in Figure 4 Panel A, no reliable increases in plasma caffeine concentration in any condition were observed across time, but there were significant differences between other conditions and CAFF/NIC for almost all time points ($p < 0.05$, Tukey's HSD). Some indications of decreases in plasma caffeine concentrations over time were observed when conditions were collapsed across nicotine-containing products (NIC and CAFF/NIC) and those that contained less or none (PLACEBO and CAFF/LN). Mean caffeine concentration (M) for NIC-CAFF/NIC was decreased relative to baseline ($M = 422.9$ ng/ml; standard error of the mean [SEM] = 124.7) at 120 minutes post product administration ($M = 334.7$ ng/ml; $SEM = 107.5$; n.s., Tukeys HSD), and for PLACEBO-CAFF/LN mean caffeine concentration was decreased relative to baseline ($M = 303.9$ ng/ml; $SEM = 75.5$) at 120 minutes post product administration ($M = 251.4$ ng/ml; $SEM = 64.9$, n.s., Tukeys HSD). Mean caffeine concentrations were significantly different between conditions by nicotine content status at 30 and 45 minutes post product administration (Tukey's HSD, $p < 0.05$). When time points were collapsed across all conditions, mean plasma caffeine concentration was 363.4 ng/ml ($SEM =$

Table 4.

Time course statistical analyses for all measures.

	Nicotine (N)			Caffeine (C)			Time (T)			N X C			N X T			C X T			N X C X T		
	F	P	η_p^2	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2
Physiological																					
Plasma caffeine[a]	2.5	n.s.	.074	0.5	n.s.	.017	12.0	.000	.280	2.3	n.s.	.069	2.7	.037	.080	1.6	n.s.	.049	.9	n.s.	.028
Plasma nicotine[a]	24.4	.000	.441	11.2	.002	.265	19.2	.000	.382	3.9	n.s.	.113	13.0	.000	.295	4.8	.003	.133	2.8	.042	.083
Heart rate[b]	5.7	.023	.155	3.1	n.s.	.090	55.2	.000	.640	3.8	n.s.	.109	3.3	.000	.097	2.0	.020	.060	1.4	n.s.	.043
Systolic BP[b]	13.5	.001	.304	2.2	n.s.	.066	2.2	.001	.067	1.8	n.s.	.053	.9	n.s.	.028	1.6	.030	.050	1.1	n.s.	.033
Diastolic BP[b]	8.4	.007	.214	5.6	.024	.153	2.8	.000	.082	4.5	.042	.127	2.0	.003	.060	1.5	n.s.	.045	.9	n.s.	.028
Expired air CO[c]	43.7	.000	.610	4.5	.043	.138	99.7	.000	.781	1.4	n.s.	.046	45.2	.000	.617	4.3	.040	.132	2.2	n.s.	.072
DES[a]																					
Nauseous	.2	n.s.	.006	0.5	n.s.	.015	3.5	.005	.101	6.0	.020	.163	1.3	n.s.	.040	.7	n.s.	.022	1.1	n.s.	.035
Dizzy	.7	n.s.	.023	0.8	n.s.	.027	6.0	.000	.161	3.1	n.s.	.091	1.0	n.s.	.032	.7	n.s.	.021	2.5	n.s.	.073
Lightheaded	3.4	n.s.	.098	0.7	n.s.	.024	14.4	.000	.317	3.3	n.s.	.097	2.0	n.s.	.062	.5	n.s.	.015	2.1	n.s.	.063
Nervous	.1	n.s.	.002	1.3	n.s.	.041	3.6	.028	.105	4.4	.045	.124	.8	n.s.	.025	.6	n.s.	.019	2.2	n.s.	.066
Sweaty	3.4	n.s.	.099	2.5	n.s.	.073	0.8	n.s.	.026	.8	n.s.	.024	.1	n.s.	.004	.4	n.s.	.013	1.4	n.s.	.043
Headache	1.0	n.s.	.031	0.8	n.s.	.024	3.7	.006	.107	3.6	n.s.	.105	.8	n.s.	.025	1.3	n.s.	.041	1.1	n.s.	.035
Salivation	.2	n.s.	.005	0.0	n.s.	.001	3.3	.017	.097	1.4	n.s.	.043	.9	n.s.	.029	.4	n.s.	.012	1.3	n.s.	.040
Heart pounding	.2	n.s.	.006	3.3	n.s.	.095	3.1	.033	.092	2.5	n.s.	.075	.4	n.s.	.014	2.2	n.s.	.067	.8	n.s.	.025
Confused	.0	n.s.	.001	0.6	n.s.	.018	4.2	.020	.119	3.6	n.s.	.103	.9	n.s.	.028	1.0	n.s.	.032	1.1	n.s.	.036
Weak	.3	n.s.	.009	0.1	n.s.	.002	3.6	.010	.104	3.0	n.s.	.089	.9	n.s.	.029	0.3	n.s.	.009	2.2	n.s.	.067
Hungry	.0	n.s.	.001	0.0	n.s.	.001	12.3	.000	.284	3.9	n.s.	.111	1.8	n.s.	.053	.4	n.s.	.011	2.6	.031	.076
Rush	2.3	n.s.	.070	1.3	n.s.	.039	10.9	.000	.260	4.8	.035	.135	2.4	.040	.072	.9	n.s.	.028	1.6	n.s.	.050
High	6.4	.017	.170	0.8	n.s.	.024	15.5	.000	.333	4.3	.046	.122	2.3	n.s.	.068	1.1	n.s.	.034	2.3	n.s.	.069
Feel drug effects	2.7	n.s.	.079	0.8	n.s.	.026	19.2	.000	.382	3.9	n.s.	.111	1.6	n.s.	.050	1.2	n.s.	.038	2.0	n.s.	.062
Like drug effects	.6	n.s.	.020	0.2	n.s.	.005	21.2	.000	.406	2.0	n.s.	.060	1.8	n.s.	.056	1.0	n.s.	.033	3.1	.010	.090
Dislike drug effects	.3	n.s.	.008	0.3	n.s.	.010	7.1	.000	.185	.2	n.s.	.008	.9	n.s.	.027	.7	n.s.	.021	1.1	n.s.	.034
Good drug effects	2.4	n.s.	.072	0.0	n.s.	.000	23.1	.000	.427	1.3	n.s.	.041	2.4	n.s.	.071	.6	n.s.	.020	1.6	n.s.	.048
Bad drug effects	1.2	n.s.	.038	0.6	n.s.	.020	8.2	.000	.210	3.4	n.s.	.098	1.0	n.s.	.030	2.5	.035	.074	1.0	n.s.	.031

	Nicotine (N)			Caffeine (C)			Time (T)			N X C			N X T			C X T			N X C X T		
	F	P	η_p^2	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2	F	p	η_p^2
POMS[a]																					
Tension	.3	n.s.	.008	1.0	n.s.	.030	3.5	.014	.100	3.2	n.s.	.093	1.1	n.s.	.034	3.6	.002	.104	1.0	n.s.	.032
Depression	1.1	n.s.	.035	.2	n.s.	.006	2.4	n.s.	.071	1.5	n.s.	.045	.7	n.s.	.023	4.2	.010	.118	.8	n.s.	.026
Anger	1.4	n.s.	.044	.1	n.s.	.002	2.4	n.s.	.072	4.1	n.s.	.118	.6	n.s.	.020	2.3	n.s.	.070	.7	n.s.	.021
Vigor	.2	n.s.	.006	.0	n.s.	.001	6.1	.001	.164	.6	n.s.	.018	1.4	n.s.	.043	1.3	n.s.	.040	.5	n.s.	.017
Fatigue	.8	n.s.	.024	.0	n.s.	.000	2.2	n.s.	.067	1.7	n.s.	.051	.7	n.s.	.021	.3	n.s.	.010	.3	n.s.	.010
Confusion	.2	n.s.	.008	1.6	n.s.	.050	2.9	n.s.	.085	1.6	n.s.	.048	1.5	n.s.	.047	1.6	n.s.	.049	1.2	n.s.	.039
Arousal	.4	n.s.	.012	.0	n.s.	.000	3.7	.016	.107	.8	n.s.	.025	.4	n.s.	.014	.5	n.s.	.016	.4	n.s.	.013
PANAS[a]																					
Positive	.0	n.s.	.000	.1	n.s.	.004	3.7	.011	.108	.1	n.s.	.002	.4	n.s.	.013	.5	n.s	.016	1.0	n.s.	.030
Negative	.9	n.s.	.027	.4	n.s.	.013	3.6	.020	.103	.8	n.s.	.025	.1	n.s.	.003	3.1	0.018	.091	1.3	n.s.	.041

[a] N = 32: $df_{Nicotine (N)}$ = (1,31); $df_{Caffeine (C)}$ = (1,31); $df_{Time (T)}$ = (7,217); $df_{N X C}$ = (1,31); $df_{N X T}$ = (7,217); $df_{C X T}$ = (7,217); $df_{N X C X T}$ = (7,217) .

[b] N = 32: $df_{Nicotine (N)}$ = (1,31); $df_{Caffeine (C)}$ = (1,31); $df_{Time (T)}$ = (24,744); $df_{N X C}$ = (1,31); $df_{N X T}$ = (24,744); $df_{C X T}$ = (24,744); $df_{N X C X T}$ = (24,744).

[c] N = 29: $df_{Nicotine (N)}$ = (1,28); $df_{Caffeine (C)}$ = (1,28); $df_{Time (T)}$ = (4,112); $df_{N X C}$ = (1,28); $df_{N X T}$ = (4,112); $df_{C X T}$ = (4,112); $df_{N X C X T}$ = (4,112).
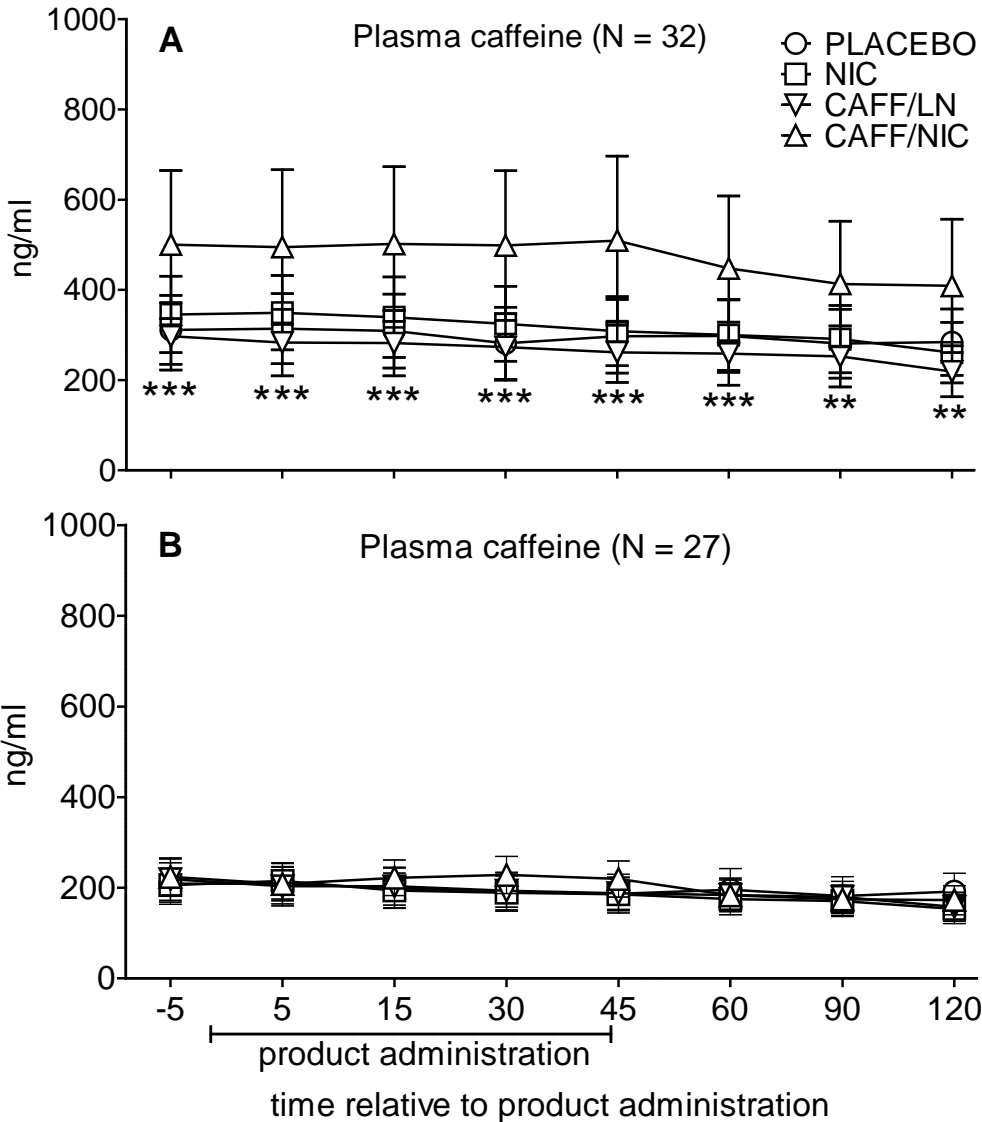
Figure 4. Mean data (±1 *SEM*) for plasma caffeine across conditions (PLACEBO = no caffeine/ no nicotine; NIC = nicotine/no caffeine; CAFF/LN = low nicotine/caffeine; CAFF/NIC = caffeine/nicotine). Panel A includes all completers (N = 32) and Panel B only includes individuals with a plasma caffeine concentration below 1000 ng/ml at baseline N = 27). Filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference relative to CAFF/NIC at that time point ($p < 0.05$, Tukey's HSD).

100.1) at baseline, 344.0 ng/ml (*SEM* = 103.1) at 45 minutes (termination of product

administration), and 293.0 (*SEM* = 86.2) at 120 minutes (end of session).

Inspection of the raw data revealed that five individuals had a baseline plasma caffeine

concentration above 1000 ng/ml during at least one session. Other clinical examinations indicate

after 24 hours of caffeine abstinence all or most individuals' plasma caffeine concentrations

would be < 1 mg/L (1000 ng/ml; Jacobson et al., 1994; Zheng & Williams, 2002), and after 12

hours approximately 75% of individuals would be under 1000 ng/ml (Majd-Ardekani et al.,

2000). Using this conservative plasma caffeine concentration cut-off (1000 ng/ml), those five

individuals were removed from the dataset and plasma caffeine data were re-analyzed (N = 27).

Results mirrored the analysis of 32 participants with a significant main effect of time (F[7,182] =

10.1, $p < 0.001$), but without a significant interaction of nicotine by time (F[7, 182] = 1.1. p =

0.355). Figure 4 Panel B displays the time course of plasma caffeine concentrations by condition

for individuals with a baseline plasma caffeine concentration less than 1000 ng/ml.

For plasma nicotine, a significant nicotine by caffeine by time interaction was observed

(p = 0.04). As seen in Figure 5, relative to baseline (collapsed across all conditions, 2.1 ng/ml,

SEM = 0.1) CAFF/NIC and NIC were associated with significant increases in plasma nicotine

level at most time points ($p < 0.05$, Tukey's HSD). The greatest mean increase for CAFF/NIC

and NIC was observed at forty-five minutes post product administration: CAFF/NIC *M* = 10.4

ng/ml, *SEM* = 1.5; NIC *M* = 8.5 ng/ml, *SEM* = 1.2; $p < 0.05$, Tukey's HSD). During the

CAFF/LN condition, mean plasma nicotine concentration was elevated significantly relative to

Figure 5. Mean data (±1 *SEM*) for plasma nicotine across conditions (PLACEBO = no caffeine/ no nicotine; NIC = nicotine/no caffeine; CAFF/LN = low nicotine/caffeine; CAFF/NIC = caffeine/nicotine; N = 32). Filled symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a significant difference relative to CAFF/NIC at that time point ($p <$ 0.05, Tukey's HSD).

baseline at 30 minutes *(M* = 5.7, *SEM* = 0.8) and 45 minutes *(M* = 5.8, *SEM* = 0.7) post product administration ($p < 0.05$, Tukey's HSD). In contrast, plasma nicotine concentration did not increase significantly during PLACEBO. Relative to CAFF/NIC plasma nicotine concentrations post product administration, all PLACEBO concentrations and CAFF/LN concentrations at 45 and 60 minutes were lower significantly ($p < 0.05$, Tukey's HSD).

For heart rate, Table 4 shows that a significant nicotine by time and caffeine by time interaction were observed (Fs > 1.9, ps < 0.05). Figure 6 displays the results across conditions and significant increases relative to baseline were observed during all conditions except for PLACEBO, and PLACEBO was the only condition where significant differences relative to CAFF/NIC were observed (ps <0.05, Tukey's HSD). When conditions were collapsed across nicotine-containing products (NIC and CAFF/NIC) and those that contained less or none (PLACEBO and CAFF/LN), the greatest mean increase relative to baseline (collapsed across all conditions, 70.4 bpm) occurred during conditions that contained nicotine at 10 minutes post product administration *(M* = 79.6 bpm, *SEM* = 1.8; $p < 0.05$; Tukey's HSD), and there were no significant differences between product types at any time point. When conditions were collapsed across caffeine-containing products (CAFF/NIC and CAFF/LN) and those that contained none (PLACEBO and NIC), the greatest mean increase relative to baseline occurred during conditions that contained caffeine at 10 minutes post product administration *(M* = 78.9, *SEM* = 1.8; $p <$ 0.05, Tukey's HSD), and there were no significant differences between product types at any time point.
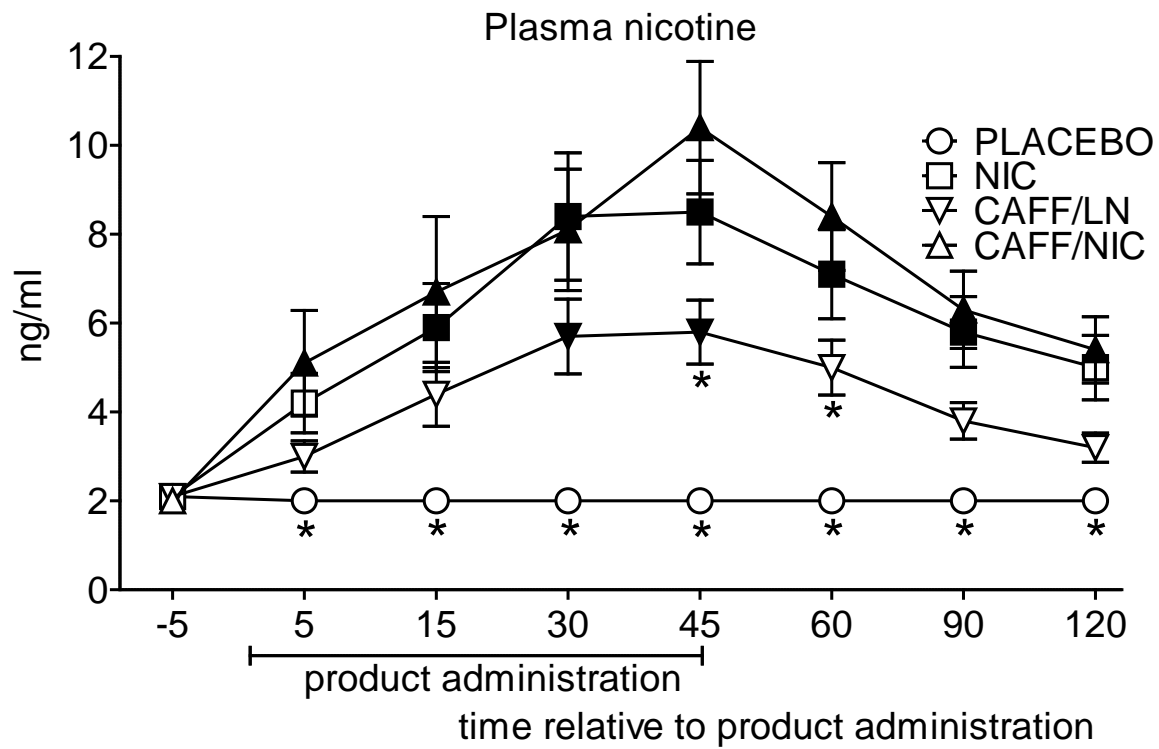
Figure 6. Mean data (±1 *SEM*) for HR across conditions (PLACEBO = no caffeine/ no nicotine;

NIC = nicotine/no caffeine; CAFF/LN = low nicotine/caffeine; CAFF/NIC = caffeine/nicotine; N

= 32). Filled symbols indicate a significant difference relative to baseline, and asterisks (*)

indicate a significant difference relative to CAFF/NIC at that time point ($p < 0.05$, Tukey's

HSD).

As Table 4 shows, a significant caffeine by time interaction was observed for systolic blood pressure and a significant nicotine by time interaction was observed for diastolic blood pressure (Fs > 1.5 ps < 0.05). Figure 7 Panel A displays systolic BP data across conditions, and there were no significant differences relative to baseline or to CAFF/NIC (n.s., Tukey's HSD). When conditions were collapsed across caffeine-containing products (CAFF/NIC and CAFF/LN) and those that contained none (PLACEBO and NIC), the greatest mean systolic blood pressure increase relative to baseline (collapsed across all conditions, 116.1 mm Hg) occurred during conditions that contained caffeine at 45 minutes post product administration ($M = 122.6$ mm Hg, $SEM = 2.5$; n.s., Tukey's HSD), and there were no significant differences relative to baseline or between product types at any time point (n.s., Tukey's HSD). Figure7 Panel B displays diastolic BP across conditions, and while there were no significant differences relative to baseline for any condition, there were two time points during PLACEBO that were significantly different relative to CAFF/NIC (ps < 0.05, Tukey's HSD). For diastolic BP, when conditions were collapsed across nicotine-containing products (NIC and CAFF/NIC) and those that contained less or none (PLACEBO and CAFF/LN), the greatest mean diastolic blood pressure increase relative to baseline (collapsed across all conditions, 64.0 mm Hg) occurred during conditions that contained nicotine at 55 minutes post product administration ($M = 70.1$ mm Hg, $SEM = 1.8$; see Figure 7 Panel B; n.s., Tukey's HSD) and there were no significant differences relative to baseline or between product types at any time point (n.s., Tukey's HSD).
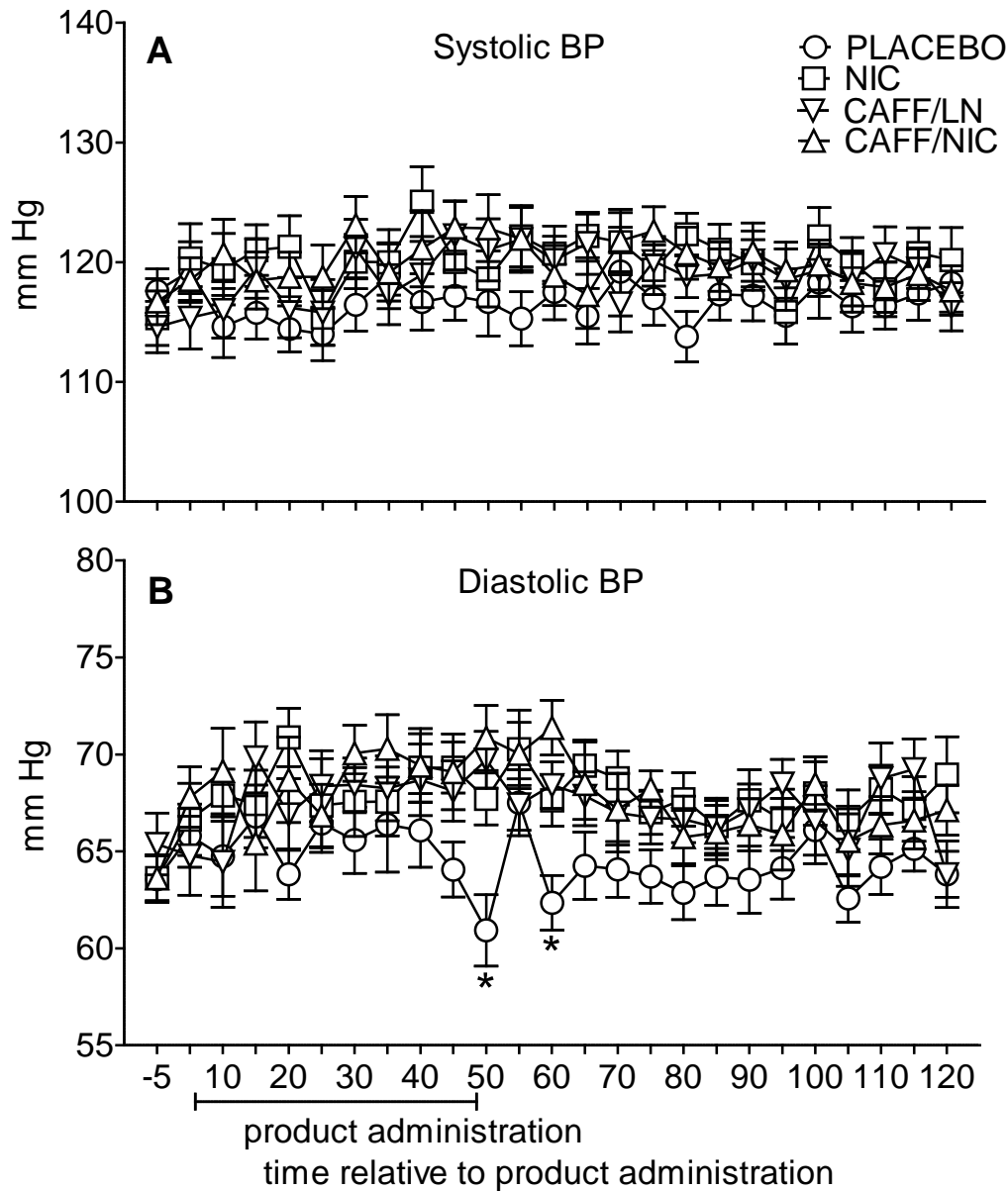
Figure 7. Mean data (±1 *SEM*) for systolic BP (Panel A; N = 32) and diastolic BP (Panel B; N =
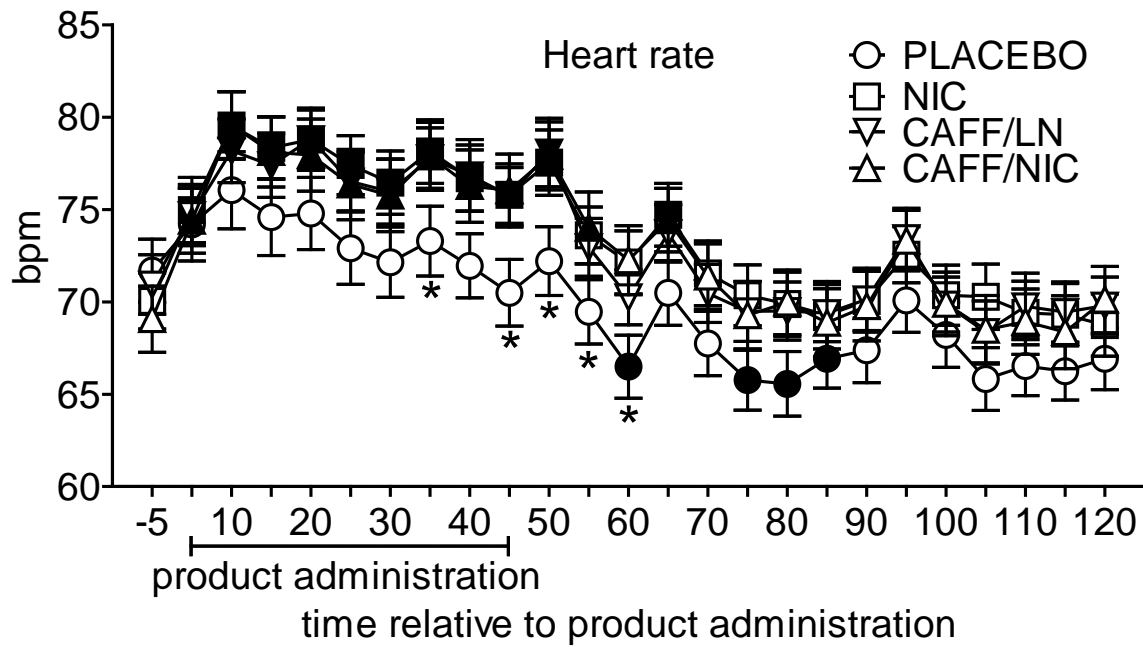
32) across conditions (PLACEBO = no caffeine/ no nicotine; NIC = nicotine/no caffeine;

CAFF/LN = low nicotine/caffeine; CAFF/NIC = caffeine/nicotine). No significant differences

relative to baseline were observed, and asterisks (*) indicate a significant difference relative to

CAFF/NIC at that time point ($p < 0.05$, Tukey's HSD).

For expired air CO, significant nicotine by time and caffeine by time interactions were observed (Fs > 4.2, ps < 0.05). Figure 8 displays mean expired air CO data across conditions and significant increases relative to baseline were observed in all conditions as well as significant differences for PLACEBO and CAFF/LN relative to CAFF/NIC (ps < 0.05, Tukey's HSD). When conditions were collapsed across nicotine-containing products (NIC and CAFF/NIC) and those that contained less or none (PLACEBO and CAFF/LN), both product groups displayed significant increases relative to baseline (all ps <0.05, Tukey's HSD). The greatest mean expired air CO increase relative to baseline (collapsed across all conditions, 2.9 ppm) occurred during conditions that contained less or no nicotine at 50 minutes post product administration (M = 29.3 ppm , SEM = 3.3; p <0.05, Tukey's HSD), and all time points post product administration were significantly higher for conditions that contained less or no nicotine (PLACEBO and CAFF/LN) compared to those containing higher amounts of nicotine (NIC and CAFF/NIC; all ps < 0.05, Tukey's HSD). When expired air CO results were examined by caffeine content status, both product types produced significant increases in expired air CO relative to baseline (all ps <0.05, Tukey's HSD), but there were no differences in mean expired air CO between caffeine containing products (CAFF/NIC and CAFF/LN) and those that did not (PLACEBO and NIC) at any time point (n.s., Tukey's HSD).

Subjective measures.

Direct effects scale. As shown in Table 4, significant nicotine by caffeine by time interactions were observed for the DES items assessing "Hungry" and "Do you like the drug effects?" (Fs > 2.5, ps < 0.05), a significant nicotine by time interaction was observed for

85
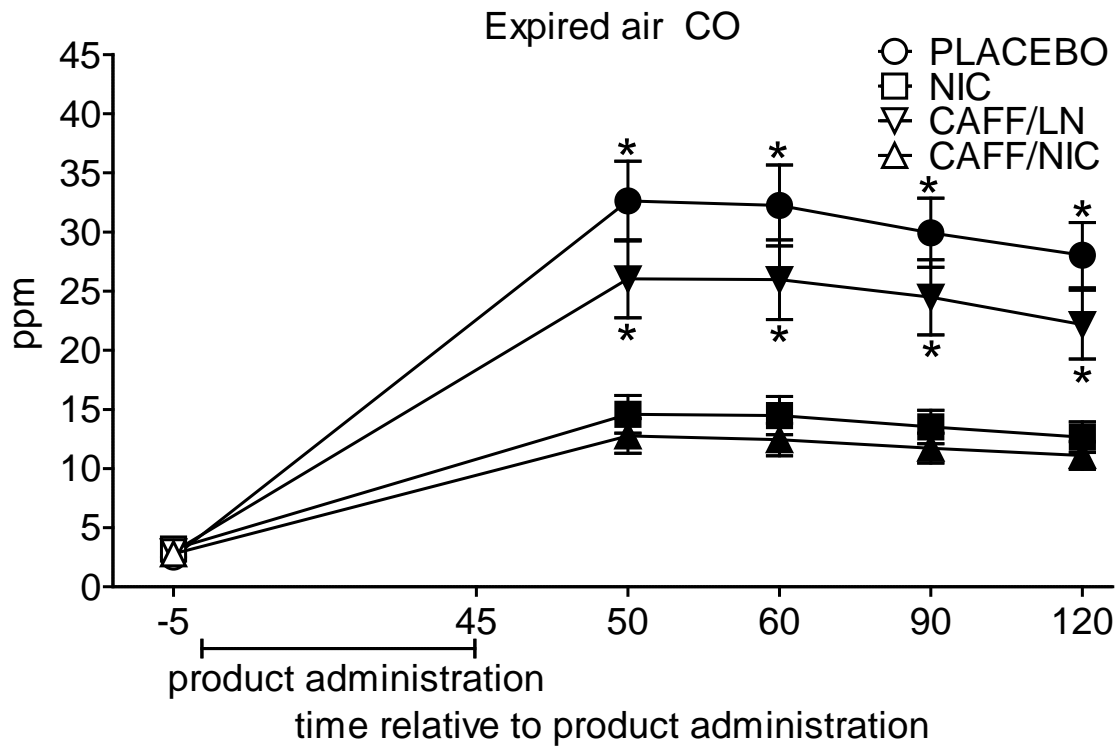
Figure 8. Mean data (±1 *SEM*) for expired air CO across conditions (PLACEBO = no caffeine/

no nicotine; NIC = nicotine/no caffeine; CAFF/LN = low nicotine/caffeine; CAFF/NIC =

caffeine/nicotine; N = 29). Filled symbols indicate a significant difference relative to baseline,

and asterisks (*) indicate a significant difference relative to CAFF/NIC at that time point ($p <$

0.05, Tukey's HSD).

"Do you feel a rush?", and a significant caffeine by time interaction was observed for "Do you feel any bad drug effects?" ($Fs > 2.3$, $ps < 0.05$). Figure 9 Panel A displays the results for "Do you like the drug effects?" across conditions an item which significant differences relative to baseline (collapsed across conditions, 2.4) were observed in all conditions following product administration until the 90 minute time point ($p < 0.05$, Tukey's HSD). The greatest mean increase relative to baseline occurred during NIC at 15 minutes post product administration ($M = 31.4$, $SEM = 4.5$), and there were no differences between CAFF/NIC and other conditions at any time point (n.s., Tukey's HSD). For the item "Hungry", significant increases relative to baseline were observed at the 90 minute time point for PLACEBO and at the 120 minute time point for all conditions except for CAFF/LN (see Figure 9 Panel B; $p < 0.05$, Tukey's HSD). There were no significant differences relative to the CAFF/NIC condition at any time point.

Results for the "Do you feel a rush?" item are displayed in Figure 10 Panel A. Across conditions, significant increases relative to baseline were observed the smoking administration period during all conditions ($p < 0.05$, Tukey's HSD), but there were no significant differences relative to CAFF/NIC (n.s., Tukey's HSD). When results were collapsed across nicotine-containing products (NIC and CAFF/NIC) and those that had little or none (PLACEBO and CAFF/LN), significant increases relative to baseline (collapsed across all conditions; $M = 1.7$, $SEM = 1.0$) were observed at 15 ($M = 15.3$, $SEM = 3.6$), 30 ($M = 17.7$, $SEM = 3.8$), and 45 ($M = 17.2$, $SEM = 4.3$) minutes for NIC-CAFF/NIC and at 30 ($M = 12.7$, $SEM = 3.0$) and 45 ($M = 15.8$, $SEM = 3.9$) minutes for PLACEBO-CAFF/LN ($ps < 0.05$, Tukey's HSD). There were no differences between product types at any time point. Results for the item "Do you feel any bad
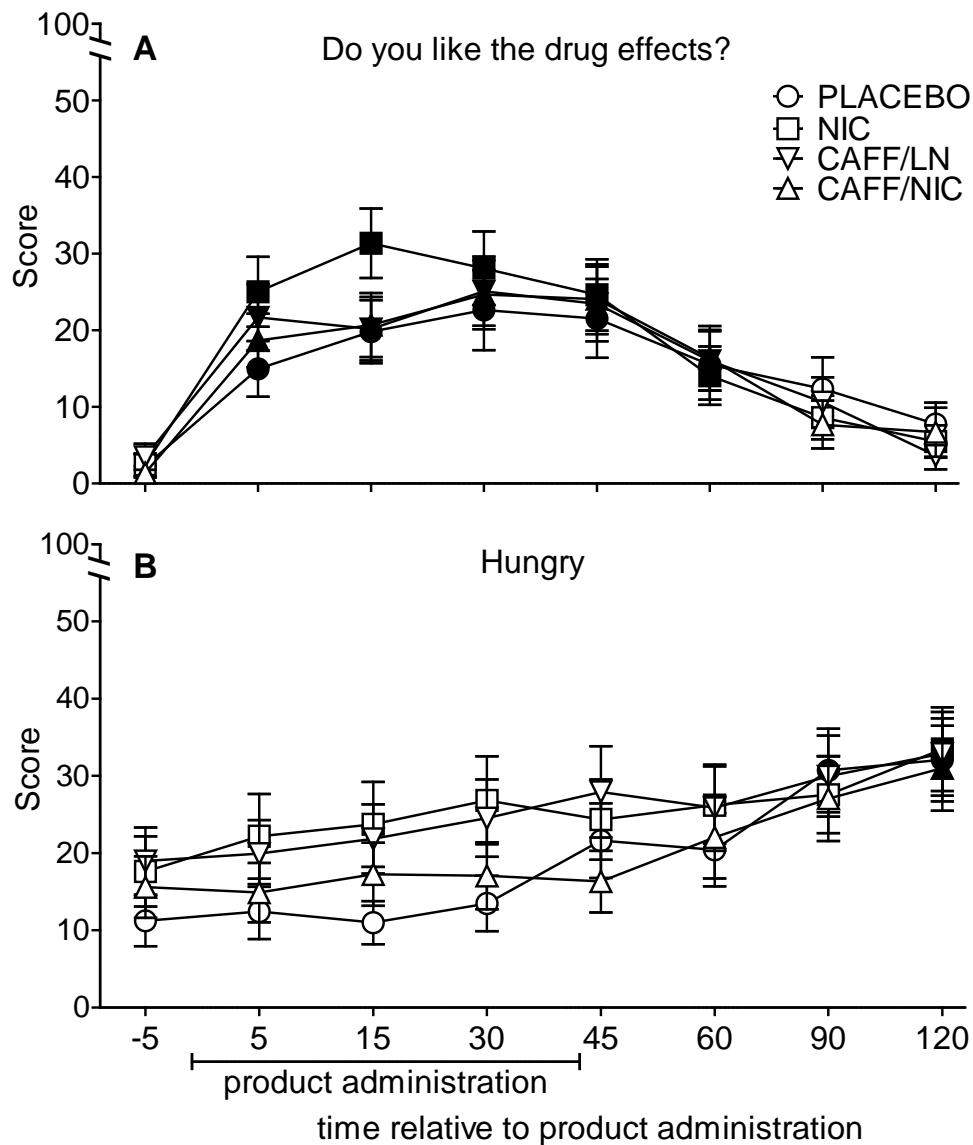
Figure 9. Mean scores (±1 *SEM*) for "Do you like the drug effects?" (Panel A; N = 32) and

"Hungry" (Panel B) across conditions (PLACEBO = no caffeine/ no nicotine; NIC = nicotine/no

caffeine; CAFF/LN = low nicotine/caffeine; CAFF/NIC = caffeine/nicotine; N = 32). Filled

symbols indicate a significant difference relative to baseline, and asterisks (*) indicate a

significant difference relative to CAFF/NIC at that time point ($p < 0.05$, Tukey's HSD).
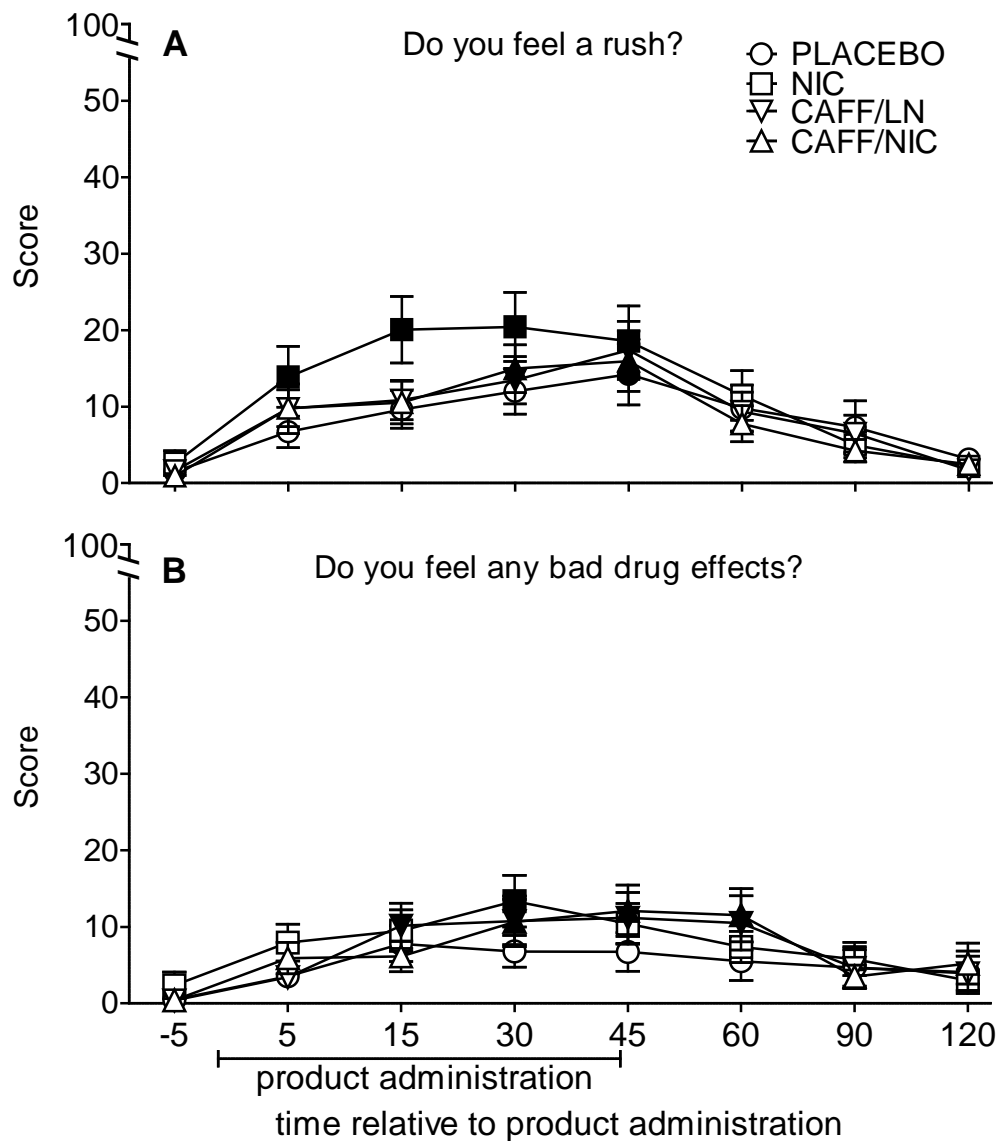
Figure 10. Mean scores (±1 *SEM*) for "Do you feel a rush?" (Panel A) and "Do you feel any bad

drug effects?" (Panel B) across conditions (PLACEBO = no caffeine/ no nicotine; NIC =

nicotine/no caffeine; CAFF/LN = low nicotine/caffeine; CAFF/NIC = caffeine/nicotine; N = 32).

Filled symbols indicate a significant difference relative to baseline ($p < 0.05$, Tukey's HSD), and

asterisks (*) indicate a significant difference relative to CAFF/NIC at that time point ($p < 0.05$,

Tukey's HSD).

drug effects?"are displayed in Figure 10 Panel B, and significant increases relative to baseline were observed during NIC, CAFF/LN and CAFF/NIC. When this item was examined by caffeine content status, significant increases relative to baseline (collapsed across conditions, 0.9) were observed at 30 ($M = 10.7$, $SEM = 3.1$), 45 ($M = 11.6$, $SEM = 3.3$), and 60 ($M = 11.0$, $SEM = 3.5$) minutes post product administration during conditions that contained caffeine (CAFF/NIC and CAFF/LN; ps < 0.05; Tukey's HSD). There were no differences between product types for any time point.

  The profile of mood states and positive and negative affect scale. The POMS factors tension/anxiety and depression/dejection and the PANAS negative affect factor had significant caffeine by time interactions (Fs > 3.0, ps < 0.05). When depression/dejection (factor with the largest F-value; see Figure 11) was examined across conditions, no significant differences relative to baseline or to CAFF/NIC at any time point were observed (n.s., Tukey's HSD). When results for this item were collapsed across caffeine content status, there were no significant differences relative to baseline for either product group. The greatest increase relative to baseline occurred during caffeine-containing conditions (CAFF/NIC and CAFF/LN) at 45 minutes ($M = 2.3$, $SEM = 0.8$) post product administration (n.s., Tukey's HSD). There were no differences between product types for any time point (n.s., Tukey's HSD). Results for POMS-tension/anxiety and PANAS-NA displayed a near identical pattern when collapsed across caffeine content status with the highest increases relative to baseline occurring during caffeine-containing conditions.

POMS-depression/dejection
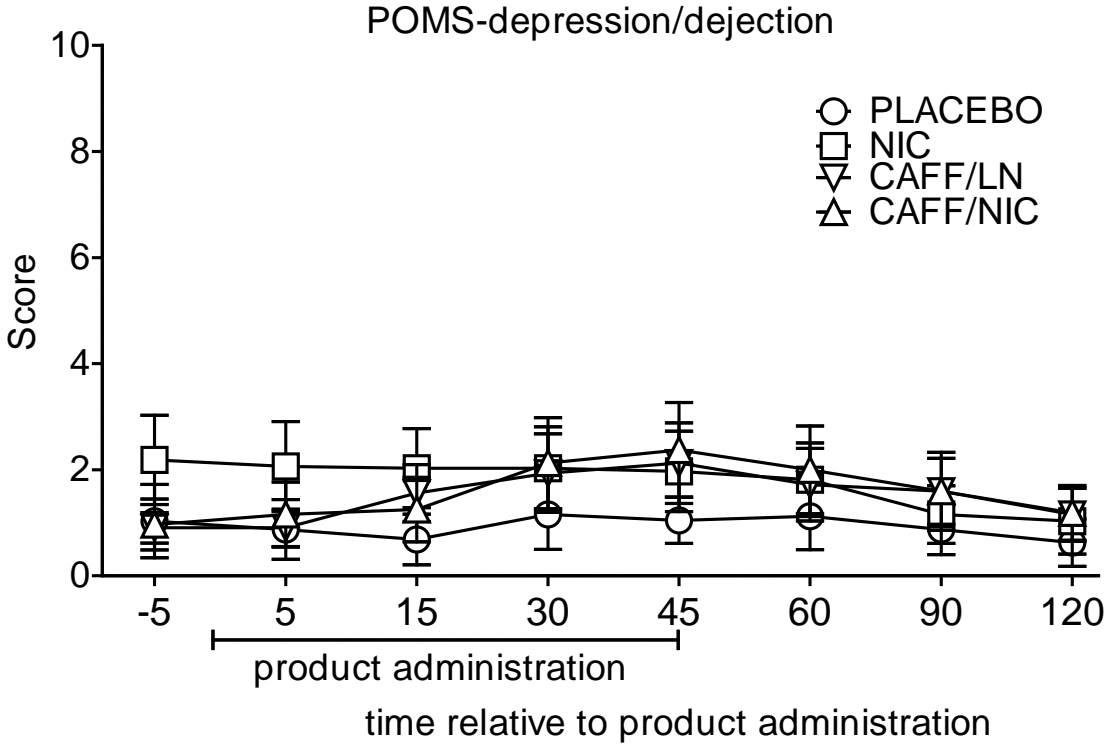
Figure 11. Mean scores (±1 *SEM*) for the POMS-Depression/Dejection factor across conditions (PLACEBO = no caffeine/ no nicotine; NIC = nicotine/no caffeine; CAFF/LN = low nicotine/caffeine; CAFF/NIC = caffeine/nicotine; N=32). No significant differences relative to baseline or to CAFF/NIC at any time point were observed (n.s., Tukey's HSD).

Peak Change Analyses

Table 5 displays peak change statistical analyses (main effects and interactions) for all measures. Interactions that involve both nicotine and caffeine factors are the most relevant as they indicate that the results observed differed between nicotine and caffeine-containing products.

Physiological measures. As Table 5 shows there were no significant peak change effects for plasma caffeine and there was a significant main effect of nicotine and a significant main effect of caffeine for plasma nicotine (Fs > 13.5, ps <0.01). Plasma nicotine mean peak change across conditions is displayed in Figure 12 and PLACEBO and CAFF/LN were significantly lower than CAFF/NIC (ps < 0.05, Tukey's HSD). When peak plasma nicotine levels were collapsed into conditions that contained nicotine (NIC and CAFF/NIC) and those that contained less or none (PLACEBO and CAFF/LN), mean peak change of plasma nicotine was approximately 4 times higher during NIC-CAFF/NIC (M = 8.7, SEM = 1.7) compared to PLACEBO-CAFF/LN (M = 2.4, SEM = 0.4; p < 0.05, Tukey's HSD). When plasma nicotine levels were collapsed into conditions that contained caffeine (CAFF/NIC and CAFF/LN) and those that did not (PLACEBO and NIC), mean peak change of plasma nicotine was approximately 2 times higher during CAFF/NIC-CAFF/LN (M = 7.3, SEM = 1.4) compared to PLACEBO-NIC (M = 3.9, SEM = 0.7; p < 0.05, Tukey's HSD).

Table 5.

Peak change from baseline statistical analyses for all measures.

	Nicotine (N)			Caffeine (C)			N X C		
	F	p	η_p^2	F	P	η_p^2	F	p	η_p^2
Physiological measures									
Plasma caffeine[a]	2.4	n.s.	.072	.7	n.s.	.021	3.9	n.s.	.111
Plasma nicotine[a]	27.5	.000	.470	13.6	.001	.305	3.9	n.s.	.111
Heart rate[a]	25.7	.000	.454	8.3	.007	.210	5.8	.022	.159
Systolic BP[a]	.9	n.s.	.027	.0	n.s.	.001	7.3	.011	.191
Diastolic BP[a]	2.1	n.s.	.063	.1	n.s.	.003	.4	n.s.	.012
Expired air CO[b]	45.3	.000	.618	3.9	n.s.	.123	1.9	n.s.	.062
DES[a]									
Nauseous	.0	n.s.	.000	.0	n.s.	.001	3.3	n.s.	.095
Dizzy	1.4	n.s.	.042	.0	n.s.	.002	2.8	n.s.	.083
Lightheaded	7.7	.009	.198	.1	n.s.	.004	2.4	n.s.	.072
Nervous	2.2	n.s.	.067	.0	n.s.	.001	5.4	.027	.147
Sweaty	5.4	.027	.148	.3	n.s.	.010	2.8	n.s.	.083
Headache	.2	n.s.	.008	.6	n.s.	.020	.8	n.s.	.026
Salivation	.7	n.s.	.023	.0	n.s.	.000	1.7	n.s.	.051
Heart pounding	.1	n.s.	.004	2.6	n.s.	.078	.6	n.s.	.020
Confused	.5	n.s.	.016	1.2	n.s.	.039	3.3	n.s.	.095
Weak	.1	n.s.	.002	.4	n.s.	.012	.1	n.s.	.003
Hungry	.9	n.s.	.030	.8	n.s.	.024	.0	n.s.	.002
Rush	4.0	n.s.	.115	.6	n.s.	.018	3.8	n.s.	.110
High	8.3	.007	.210	.5	n.s.	.014	3.2	n.s.	.094
Feel drug effects	5.2	.030	.143	.9	n.s.	.029	8.4	.007	.213
Like drug effects	3.5	n.s.	.102	.3	n.s.	.009	1.7	n.s.	.051
Dislike drug effects	.6	n.s.	.020	.1	n.s.	.004	.9	n.s.	.027
Good drug effects	2.8	n.s.	.083	.4	n.s.	.014	1.7	n.s.	.053
Bad drug effects	3.0	n.s.	.089	.7	n.s.	.023	.6	n.s.	.018
POMS[a]									
Tension	2.2	n.s.	.066	8.7	.006	.219	1.4	n.s.	.042
Depression	.5	n.s.	.017	6.6	.015	.175	.8	n.s.	.026
Anger	.1	n.s.	.003	1.1	n.s.	.036	3.5	n.s.	.102
Vigor	1.3	n.s.	.040	.0	n.s.	.000	.0	n.s.	.000
Fatigue	.1	n.s.	.005	.1	n.s.	.004	.9	n.s.	.028
Confusion	.1	n.s.	.004	3.4	n.s.	.098	.3	n.s.	.009
Arousal	.1	n.s.	.002	.2	n.s.	.006	.7	n.s.	.023
PANAS[a]									
Positive	.1	n.s.	.004	0.0	n.s.	.001	.4	n.s.	.014
Negative	1.4	n.s.	.043	5.3	.028	.147	1.4	n.s.	.043

[a] N = 32: $df_{Nicotine\ (N)} = (1,31)$; $df_{Caffeine\ (C)} = (1,31)$; $df_{N\ X\ C} = (1,31)$
[b] N = 29: $df_{Nicotine\ (N)} = (1,28)$; $df_{Caffeine\ (C)} = (1,28)$; $df_{N\ X\ C} = (1,28)$

Figure 12. Mean peak change data (±1 *SEM*) for plasma nicotine across conditions (PLACEBO

= no caffeine/ no nicotine; NIC = nicotine/no caffeine; CAFF/LN = low nicotine/caffeine;

CAFF/NIC = caffeine/nicotine; N = 32). Asterisks (*) indicate a significant difference relative to

CAFF/NIC at that time point ($p < 0.05$, Tukey's HSD).

For both HR and systolic BP, a significant nicotine by caffeine interaction was observed (Fs > 5.7, ps < 0.05). Mean peak change for HR was the highest and identical for NIC and CAFF/NIC (M = 14.1, SEM = 1.3) and was followed by CAFF/LN (M = 11.6, SEM = 1.4) and PLACEBO (M = 7.1, SEM = 0.9; see Figure 13 Panel A). Mean peak change HR for PLACEBO differed significantly from CAFF/NIC (p < 0.05, Tukey's HSD). Mean peak change for systolic BP was the highest for NIC (M = 29.4, SEM = 2.6) followed by CAFF/LN (M = 26.2, SEM = 2.2), CAFF/NIC (M = 23.2, SEM = 2.6), and PLACEBO (M = 20.9, SEM = 2.7; see Figure 13 Panel B). There were no significant differences for systolic BP between other conditions and CAFF/NIC (n.s., Tukey's HSD).

For expired air CO, there was a significant main effect of nicotine. Figure 14 displays results across conditions, and PLACEBO and CAFF/LN peak change concentrations for expired air CO were significantly higher than CAFF/NIC (ps < 0.05, Tukey's HSD). When results were examined by nicotine content status, significantly higher mean peak change was observed for conditions that contained little or no nicotine (PLACEBO and CAFF/LN; M = 27.6, SEM = 3.5) compared to those contained nicotine (NIC and CAFF/NIC; M = 11.0, SEM = 1.4; p < 0.05, Tukey's HSD).

Subjective measures.

Direct effects scale. For the items "Nervous" and "Do you feel any drug effects?" a significant nicotine by caffeine interaction was observed (Fs > 5.3, ps < 0.05) and for the items "Lightheaded", "Sweaty", and "How high are you?" a main effect of nicotine was observed (Fs >5.1, ps < 0.05). For the "Do you feel any drug effects?" item (largest interaction F-value
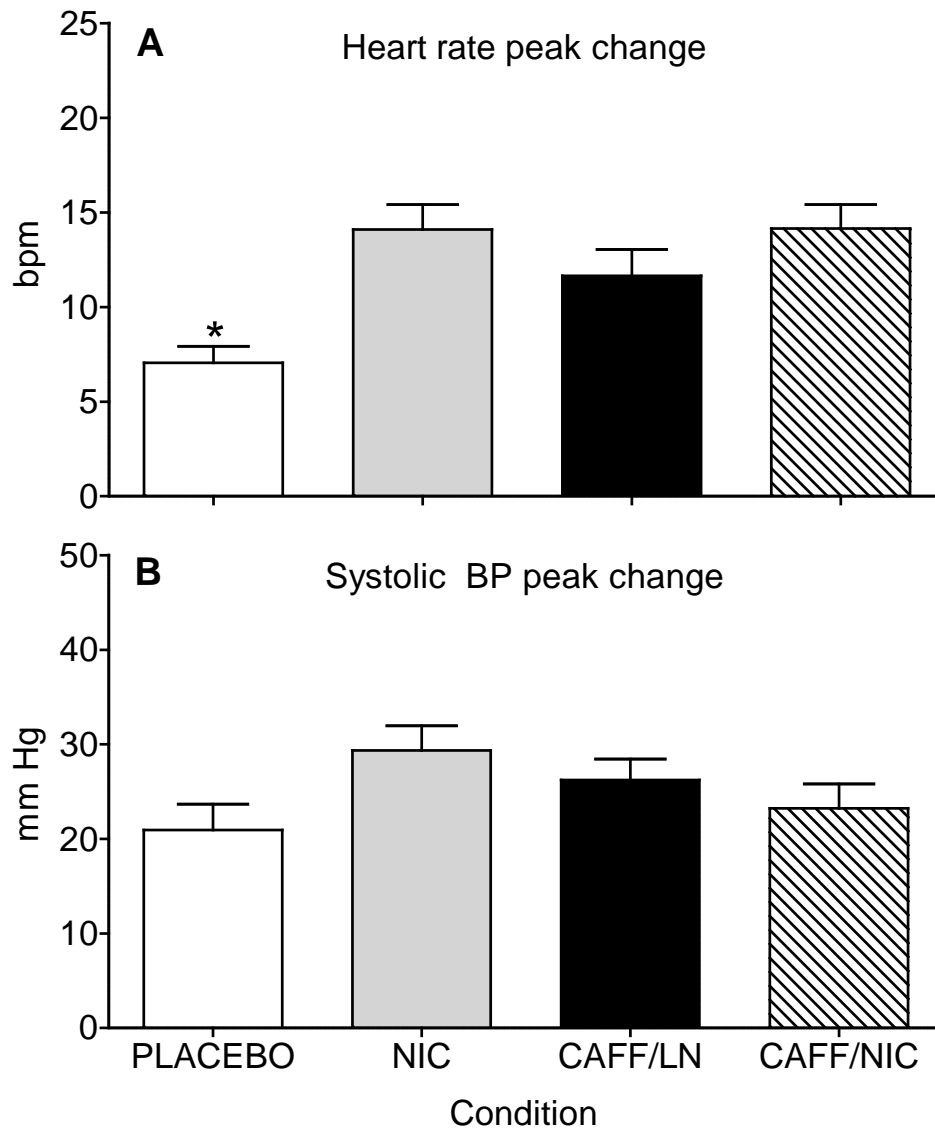
Figure 13. Mean peak change data (±1 *SEM*) for HR (Panel A; N= 32) and systolic BP (Panel B)

across conditions (PLACEBO = no caffeine/ no nicotine; NIC = nicotine/no caffeine; CAFF/LN

= low nicotine/caffeine; CAFF/NIC = caffeine/nicotine; N = 32). Asterisks (*) indicate a

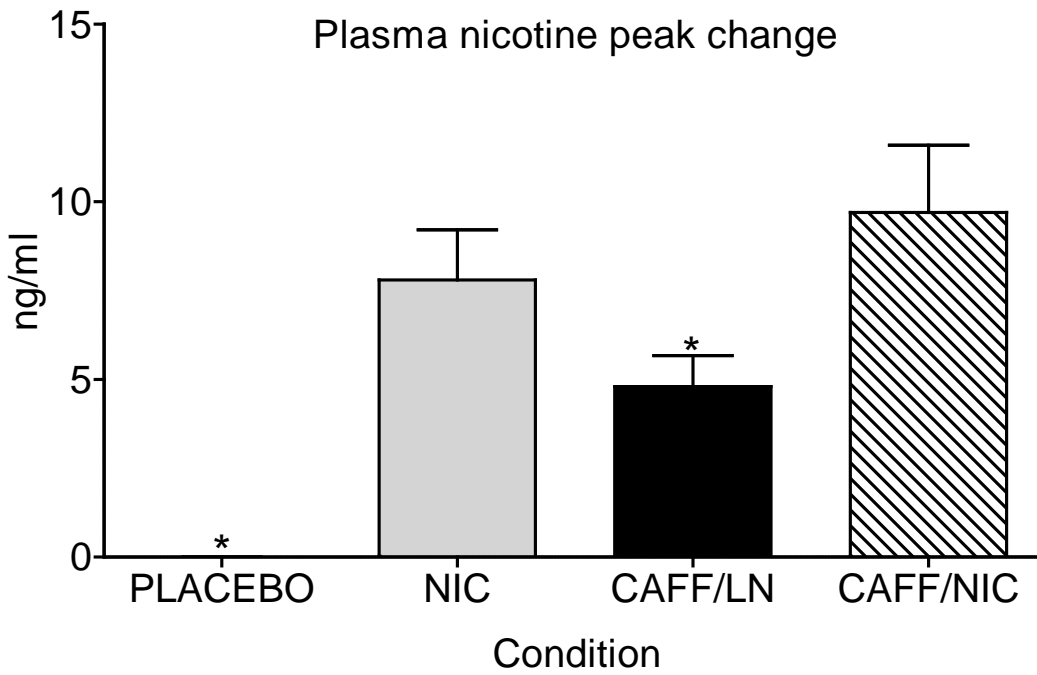significant difference relative to CAFF/NIC ($p < 0.05$, Tukey's HSD).
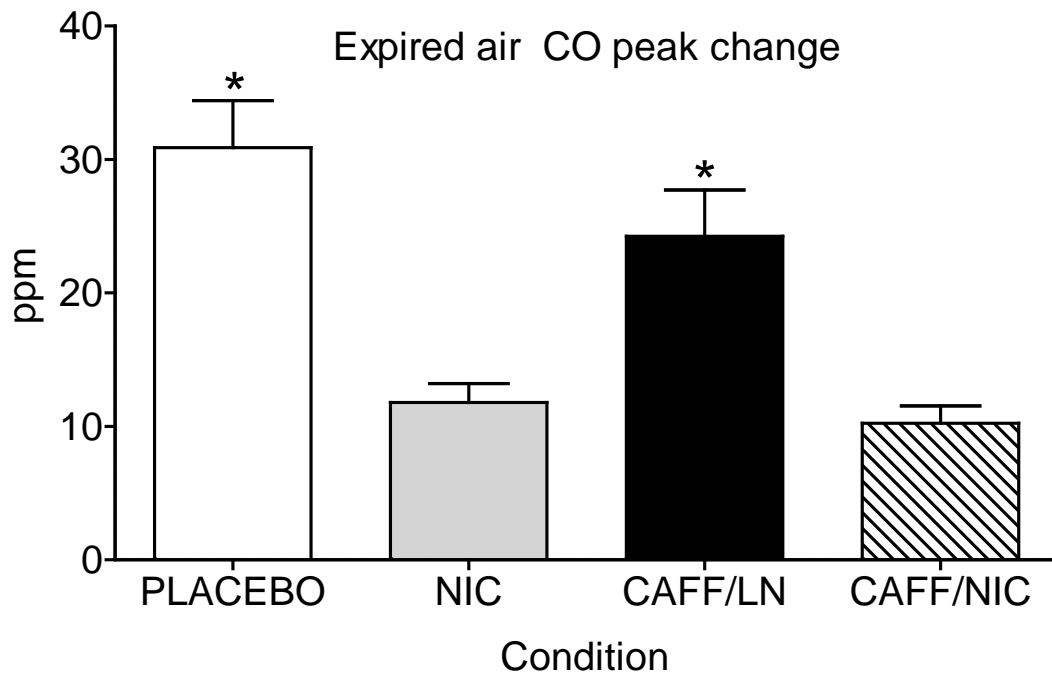
Figure 14. Mean peak change data (± 1 *SEM*) for expired air CO across conditions (PLACEBO =

no caffeine/ no nicotine; NIC = nicotine/no caffeine; CAFF/LN = low nicotine/caffeine;

CAFF/NIC = caffeine/nicotine; N= 32). Asterisks (*) indicate a significant difference relative to

CAFF/NIC ($p < 0.05$, Tukey's HSD).

observed; see Figure 15), mean peak change was the highest for NIC ($M = 39.9$, $SEM = 5.2$) followed by CAFF/NIC ($M = 31.8$, $SEM = 5.0$), CAFF/LN ($M = 28.6$, $SEM = 4.9$), and PLACEBO ($M = 26.2$, $SEM = 5.6$). There were no significant differences relative to CAFF/NIC (n.s., Tukey's HSD). Results for "Nervous" were near identical with the highest mean peak change scores observed during NIC ($M = 8.3$, $SEM = 3.0$) and the lowest during PLACEBO ($M = 1.7$, $SEM = 0.5$) with no significant differences relative to CAFF/NIC. The DES item with the largest main effect F-value for nicotine was "How high are you?", and results across conditions indicated there were no significant differences relative to CAFF/NIC (see Figure 16; n.s., Tukey's HSD). When mean peak change values were collapsed by nicotine-containing status, mean score for nicotine-containing conditions (NIC and CAFF/NIC; $M = 28.3$, $SEM = 4.7$) was higher than those that contained little or none (PLACEBO and CAFF/LN; $M = 20.5$, $SEM = 4.4$; n.s.; Tukey's HSD). This identical pattern of results was observed for the items "Lightheaded", and "Sweaty".

The profile of mood states and positive and negative affect scale. Among these two subjective measures, a significant main effect of caffeine was observed for the POMS factors tension/anxiety and depression/dejection and for the PANAS negative affect factor. The POMS factor tension/anxiety had the largest main effect F-value for caffeine, and results across conditions are displayed in Figure 17. Across conditions, peak change score for PLACEBO was significantly lower than CAFF/NIC ($p < 0.05$; Tukey's HSD). When scores for this item were collapsed by caffeine content status, mean peak change score was higher among conditions that contained caffeine (CAFF/NIC and CAFF/LN; $M = 3.2$, $SEM = 0.7$) compared those did not
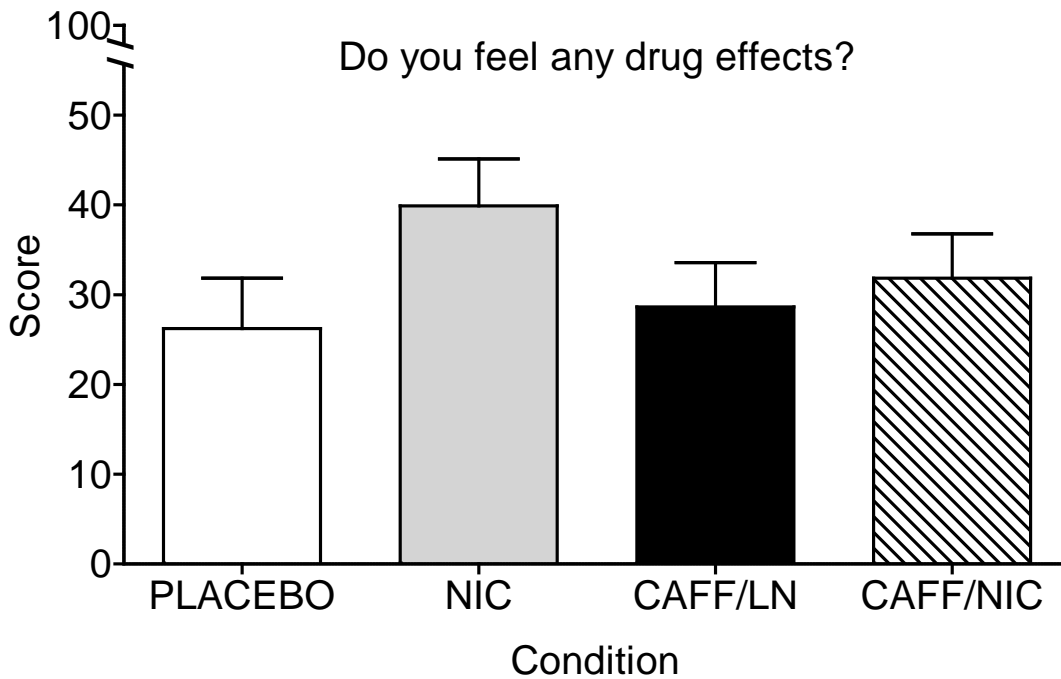
Figure 15. Mean peak change data (±1 *SEM*) for the DES item "Do you feel any drug effects?"

across conditions (PLACEBO = no caffeine/ no nicotine; NIC = nicotine/no caffeine; CAFF/LN

= low nicotine/caffeine; CAFF/NIC = caffeine/nicotine; N= 32). There were no significant

differences relative to CAFF/NIC for any condition (n.s., Tukey's HSD).
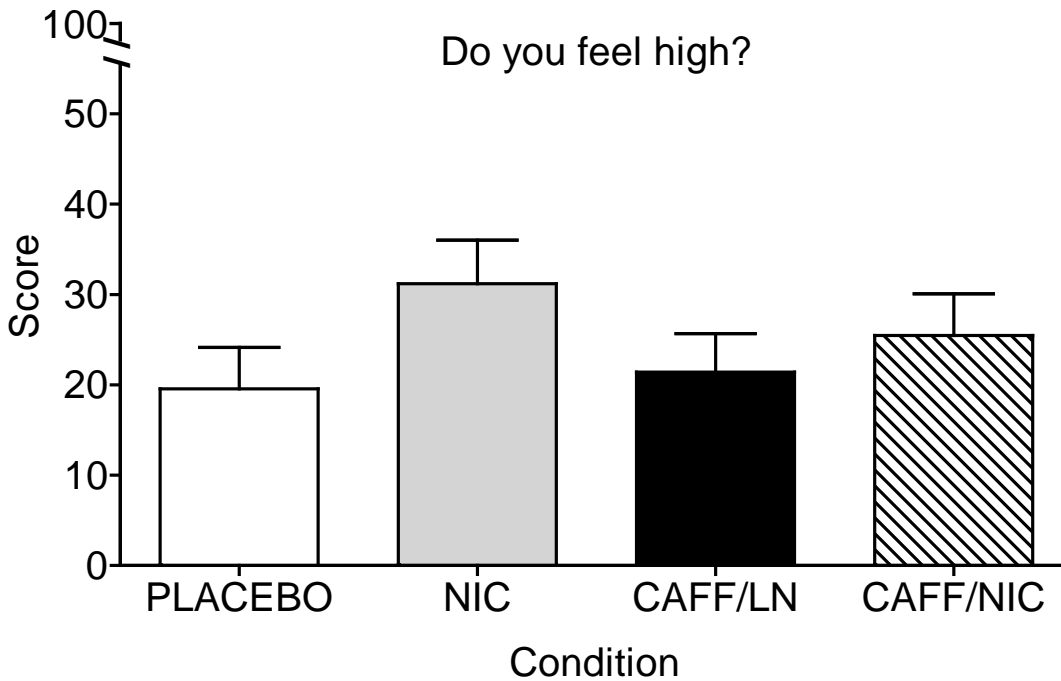
Figure 16. Mean peak change data (±1 *SEM*) for the DES item "Do you feel high?" across

conditions (PLACEBO = no caffeine/ no nicotine; NIC = nicotine/no caffeine; CAFF/LN = low

nicotine/caffeine; CAFF/NIC = caffeine/nicotine; N= 32). There were no significant differences

between conditions relative to CAFF/NIC (n.s., Tukey's HSD).
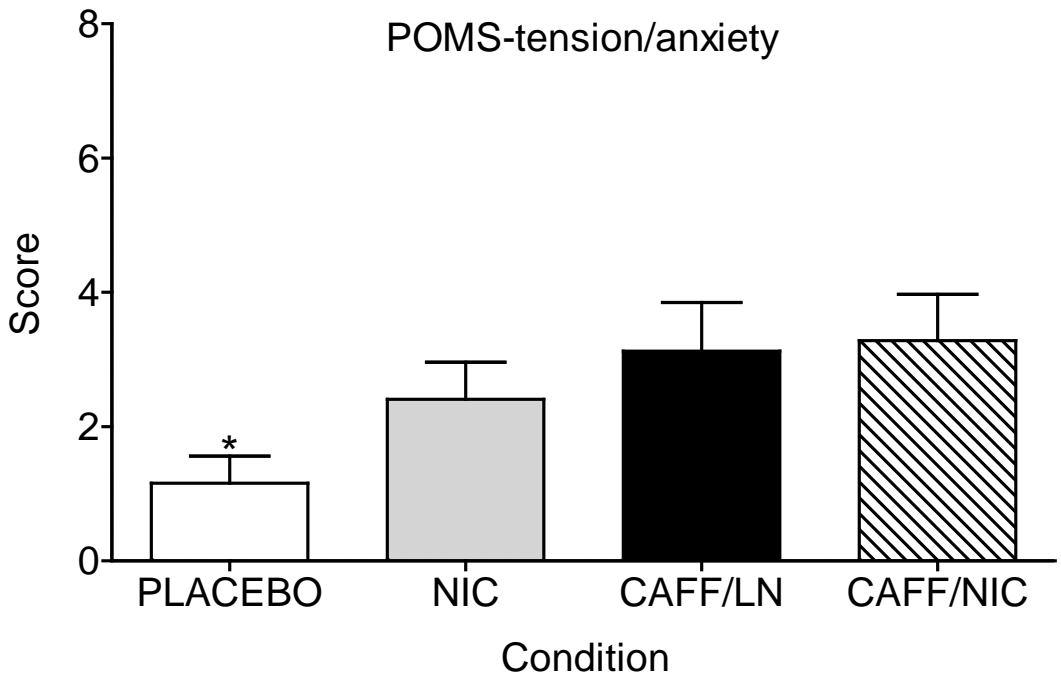
Figure 17. Mean peak change data (±1 *SEM*) for the POMS factor tension/anxiety across

conditions (PLACEBO = no caffeine/ no nicotine; NIC = nicotine/no caffeine; CAFF/LN = low

nicotine/caffeine; CAFF/NIC = caffeine/nicotine; N= 32). Asterisks (*) indicate a significant

difference relative to CAFF/NIC ($p < 0.05$, Tukey's HSD).

(PLACEBO and NIC; M = 1.8, SEM = 0.5; see Figure 17; n.s.; Tukey's HSD). Results for

POMS-depression/dejection and PANAS-NA factors displayed an identical pattern of results

when collapsed by caffeine content status with higher scores observed during conditions that

contained caffeine but not significantly different between product types (n.s., Tukey's HSD).

Puff Topography

Mean values for all puff topography measures by condition are displayed in Table 6 and

the results of statistical analyses are shown in Table 7. A significant main effect of nicotine was

observed for mean IPI, total volume, and mean puff volume, total puffing time, and puff duration

(Fs > 19.2, ps < 0.001), and a significant main effect of caffeine was observed for mean puff

volume and puff duration (Fs > 5.7 p < 0.001). When IPI was examined by nicotine status, a

shorter IPI between puffs was observed during conditions containing little or no nicotine

(PLACEBO and CAFF/LN; M = 34.1 s, standard deviation [$SD]$ = 23.4) compared to those with

nicotine (NIC and CAFF/NIC; M = 41.3 s, SD = 29.8), but this difference was not significant;

n.s.; Tukey's HSD). When total volume was examined by nicotine status, a significantly higher

total volume was observed during conditions containing little or no nicotine (PLACEBO and

CAFF/LN; M = 53.4 L, SD = 32.0) compared to those with nicotine (NIC and CAFF/NIC; M =

31.2 L, SD = 19.7; p < 0.05; Tukey's HSD).

Table 6.

Mean (standard deviation) of all topography measures.

	PLACEBO	NIC	CAFF/LN	CAFF/NIC
Mean IPI (s)	34.7 (21.8)	44.9 (33.6)	33.5 (25.0)	37.7 (26.0)
Puffs	94.8 (57.7)	94.8 (115.3)	121.4 (144.9)	104.4 (113.5)
Total volume (l)	56.8 (33.8)	31.2 (22.4)	49.9 (30.2)	31.2 (17.0)
Mean puff volume (ml)	683.1 (327.6)	422.5 (250.4)	508.0 (227.2)	369.4 (192.1)
Total puffing time (min)	4.9 (2.5)	2.9 (1.8)	4.8 (2.5)	3.1 (1.8)
Puff duration (s)	3.5 (2.0)	2.3 (.9)	3.0 (1.4)	2.2 (1.5)

Table 7.

Statistical analyses for topography measures.

	Nicotine (N)	p	η_p^2	Caffeine (C)	p	η_p^2	N X C	p	η_p^2
Mean IPI (s)	19.3	.000	.383	2.1	n.s.	.062	2.2	n.s.	.067
Puffs	2.1	n.s.	.064	2.9	n.s.	.087	.7	n.s.	.023
Total volume (l)	55.7	.000	.643	.9	n.s.	.027	1.6	n.s.	.050
Mean puff volume (ml)	43.8	.000	.585	15.3	.000	.331	2.4	n.s.	.072
Total puffing time (min)	72.7	.000	.701	.0	n.s.	.000	.4	n.s.	.011
Average puff duration (s)	36.9	.000	.543	5.8	.022	.158	1.0	n.s.	.032

Note: N = 32: $df_{Nicotine (N)}$ = (1,31); $df_{Caffeine (C)}$ = (1,31); $df_{N X C}$ = (1,31)

When mean puff volume was examined by nicotine content, higher mean puff volumes were observed during conditions that contained little or no nicotine (PLACEBO and CAFF/LN; $M = 595.5$ ml, $SD = 302.4$) compared to those with nicotine (NIC and CAFF/NIC; $M = 395.9$ ml, $SD = 221.3$; $p < 0.05$; Tukey's HSD). When mean puff volume was examined by caffeine content status, conditions containing none (PLACEBO and NIC; $M = 552.8$ ml, $SD = 289.0$) were not significantly different compared to those that contained caffeine (CAFF/NIC and CAFF/LN; $M = 438.6$ ml, $SD = 234.7$; n.s., Tukey's HSD).

Total puffing time was significantly shorter during conditions that contained nicotine (CAFF/NIC and CAFF/LN; $M = 3.0$ min, $SD = 1.9$) compared to those that contained little or none (PLACEBO and NIC; $M = 4.8$ min, $SD = 2.5$; $p < 0.05$; Tukey's HSD). Average puff duration was also significantly shorter during conditions that contained nicotine (CAFF/NIC and CAFF/LN; $M = 2.3$ s, $SD = 1.2$) compared to those that contained little or none (PLACEBO and NIC; $M = 3.3$ s, $SD = 1.7$; $p < 0.05$; Tukey's HSD). When average puff duration was examined by caffeine content status, there was not a significant difference between condition groups (n.s., Tukey's HSD).

Overview

Caffeine and nicotine are the two most commonly consumed licit psychoactive drugs in the world. In addition, their co-administration is relatively common with over 86% of cigarette smokers report using caffeine versus 77% of non-smokers (Swanson et al., 1994). Some previous research suggests the combination of nicotine and caffeine may produce effects that are more rewarding or pleasurable than either drug alone (Jones & Griffiths, 2003; Perkins et al., 1994) and this potential reward enhancement may influence patterns of tobacco use initiation and maintenance. Waterpipe tobacco smoking is an alternative tobacco use method that is increasing in prevalence in the U.S. especially among adolescent and young adult populations (Barnett Curbow, Soule, Tomar, & Thombs, 2009; Akl et al., 2011; Smith et al., 2011) and offers a novel opportunity for nicotine and caffeine co-administration via a caffeinated tobacco product: Tangiers F-Line. This product enables users, who may be among an age group that is especially vulnerable to tobacco use initiation, to consume this combination of drugs in a single form. Data concerning "smoked" (i.e., volatilized) caffeine are sparse (Zandvilet et al., 2005; Brenneisen, & Hasler, 2002), but reports indicate it is absorbed and metabolized similarly to other methods of caffeine administration. Taken with the evidence from previous nicotine and caffeine co-administration studies, this caffeinated tobacco product was hypothesized to enhance reward-related and cardiovascular effects in users relative to typical waterpipe tobacco preparations.

The purpose of this study was to compare, using a within-subject, factorial design, the subjective and cardiovascular effects of smoking caffeinated waterpipe tobacco with the effects

of smoking waterpipe preparations containing nicotine and no caffeine, low nicotine and caffeine, or neither nicotine nor caffeine. To that end, data were analyzed from thirty-two waterpipe smokers who participated in four Latin-squared ordered sessions that differed by product administered. During each session, there was a 45-minute double-blind product administration period. In addition, blood plasma was collected, cardiovascular, expired air CO, and puff topography measurements were made, and subjective measures were administered. The outcome measures of primary interest were cardiovascular (BP) and subjective effects as well as plasma caffeine exposure. As noted in the results (see Figure 4), the caffeine-containing waterpipe products failed to deliver measurable caffeine doses in this study. Therefore, the discussion below focuses primarily on (1) the effects of smoking nicotine-containing tobacco in a waterpipe, (2) potential non-pharmacological influences on waterpipe smoking behavior, (3) a future study design, and (4) limitations of the study.

Effects of Smoking Nicotine-containing Tobacco in a Waterpipe

Several results of the current study were similar to those observed in another placebo-controlled examination of smoking nicotine-containing tobacco in a waterpipe in a clinical laboratory (Blank et al., 2011). That previous study involved two double-blind counterbalanced sessions in which participants smoked their preferred flavor and brand of waterpipe tobacco or a flavor matched non-tobacco preparation for 45 minutes or longer (Blank et al., 2011). Similar to the current study, physiological (plasma nicotine, HR, BP, and expired air CO) and subjective responses were measured periodically. At the conclusion of the smoking period, the mean nicotine concentration during the placebo condition was 2.1 ng/ml (*SEM* = 0.0), and during the

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active tobacco condition was 5.6 ng/ml ($SEM = 0.7$). In the current study, the highest plasma nicotine exposure during nicotine-containing conditions was observed at the conclusion of the smoking period during CAFF/NIC ($M = 10.4$, $SEM = 1.5$) and NIC ($M = 8.5$, $SEM = 1.2$), and CAFF/LN ($M = 5.8$, $SEM = 0.7$). CAFF/NIC plasma nicotine concentrations were significantly higher than all of those observed during PLACEBO and at 45 and 60 minutes during CAFF/LN. During both placebo-controlled studies reliable increases in HR were observed during the active or tobacco-containing conditions but not during the placebo (nicotine-free) condition. No reliable BP effects were observed during the previous examination (Blank et al., 2011) or the current study.

For the subjective effect measures of the earlier study, there were few interactions of condition and time and many significant main effects of time (Blank et al., 2011). For example, significant main effects of time were observed for the items "Urges to smoke" and "Anxious" with decreases post-smoking observed during both conditions. This pattern of results indicates that these subjective effects decreased independent of the nicotine content of the product smoked. Other subjective effect items including "Dizzy", "Lightheaded", and "Was the waterpipe pleasant?" were observed to increase post-smoking with no significant differences between conditions. This pattern of results also was observed for most subjective items during the current study (see Table 4). Thus, in both the previous study (Blank et al., 2011) and the current study, experienced waterpipe tobacco smoking participants reported similar subjective effects across nicotine-containing and nicotine-free conditions.

Important differences between the above mentioned placebo-controlled waterpipe study and the current study are related to puff topography and expired air CO exposure. Table 8 displays the puff topography results for Blank et al. (2011), and when these mean values were analyzed by condition there were no significant differences. However, results for puff topography did differ between conditions in the current study (see Table 7), with a significantly smaller mean total volume and mean puff volume during NIC-CAFF/NIC compared to PLACEBO-CAFF/LN. As total smoke volume was positively correlated with expired air CO during all conditions ($r = 0.54$-0.80; N = 29, $p < 0.01$), the larger smoke volumes observed for PLACEBO-CAFF/LN may explain the higher CO concentrations observed during these conditions compared to NIC-CAFF/NIC (see Figure 8). This finding was not observed during Blank et al., (2011) where nicotine content did not influence puff topography or expired air CO concentrations. Compensation or a change in smoking behavior to adjust to the mainstream smoke nicotine yield has been observed among cigarette smokers (Baldinger, Hasenfratz, & Bättig, 1995; Guyatt, Kirkham, Mariner, Baldry, & Cumming, 1989), and may also be driving the differences in puff topography observed in this study. Interestingly, mean total puff volumes for the previous placebo controlled study (Placebo=55.7 l, Active=57.0 l; Blank et al., 2011) were more similar to conditions during the current study that contained little to no nicotine (PLACEBO=56.8; CAFF/LN=49.9) compared to conditions that contained nicotine (CAFF/NIC and NIC=31.2 l). These differences may also be associated with the higher plasma nicotine concentrations observed during the current study for CAFF/NIC and NIC. Compared to mean plasma nicotine concentrations in the active condition at the conclusion of smoking (Blank et al.,

Table 8.

Puff topography statistics for a double-blind placebo-controlled waterpipe study among current waterpipe users (Blank et al., 2011).

	Active		Placebo	
	Mean	SD	Mean	SD
Inter-puff-interval (s)	47.5	21.4	45.8	26.4
Puff number	66.3	42.2	71.2	48.6
Total puff volume (l)	57.0	45.6	55.7	32.0
Mean puff volume (ml)	906.1	517.4	873.0	366.7
Puff duration (s)	3.9	1.5	3.7	1.4

Note: Active=participant preferred waterpipe tobacco flavor/brand smoked; Placebo=flavor matched non-nicotine waterpipe product smoked (i.e., Soex).

2011), mean nicotine concentration was 2.9 ng/ml greater during NIC and 4.8 ng/ml greater

during CAFF/NIC. With reduced smoke inhalation and increased nicotine exposure compared to

this previous study (Blank et al., 2011), these results suggest that participants in this study may

have reduced their puff volumes in an attempt to compensate for the greater nicotine delivery

associated with the NIC and CAFF/NIC conditions. However, this interpretation is speculation:

with two studies reporting contradictory results, more work is needed to clarify the extent to

which behavioral compensation occurs in waterpipe tobacco smokers.

A finding specific to the current study was the observation that neither caffeinated

waterpipe tobacco product (CAFF/NIC, CAFF/LN) produced reliable increases in plasma

caffeine concentrations under the conditions utilized here. This failure to observe increases in

plasma caffeine could be due various reasons. One is that the assay used to detect caffeine

concentrations in plasma was not sensitive. Caffeine assay calibration/standard curve indicates

that this insensitivity was unlikely using the current method (see Figure 3). There is also a

chance that the caffeine used in the tobacco was a salt form (i.e., caffeine citrate) which could

have impacted volatilization capacity/delivery characteristics. All previous work examining

caffeine volatilization has been performed using an anhydrous base form of caffeine (see Klous,

Lee, Hillebrand, et al., 2006; Klous, Lee, Van den Brink, et al., 2006; Brenneisen & Hasler,

2002). Unfortunately more specific information concerning the methodology of adding caffeine

to these tobacco products was unavailable. Lastly according to chemical analysis conducted

specifically for this study, on average CAFF/NIC and CAFF/LN contained 0.5 mg/g of caffeine,

and 10 grams of product were loaded in the waterpipe head for each session. Thus, the maximal

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amount of caffeine in each head was 5 mg. Inhalation of a 100 mg caffeine tablet heated via a

lighter or heating plate has been estimated to produce plasma concentrations of caffeine in a

typical individual of 1312 ng/ml (similar to consumption of a beverage with 80 mg of caffeine,

1335 ng/ml; Zandvliet et al., 2005). If this relationship was equivalent for caffeine consumed via

inhalation of tobacco smoke (1312/100=13.12 ng/ml per mg of caffeine inhaled), the potential

plasma caffeine increase possible from the products used for the current study would have been

66 ng/ml (5*13.12 ng/ml). Thus, the small amounts of caffeine present in the caffeinated

products studied is another likely explanation for the observation that participants in this study

were not exposed to caffeine, as indexed by plasma caffeine concentrations. Importantly, this

study was sensitive enough to detect smoking-induced changes in plasma nicotine: the time

course analysis of plasma nicotine concentration indicated the η_p^2 term for the nicotine by time

interaction and main effect of nicotine were of moderate size (accounted for 30-44 % of

variance; see Table 4). In sum, there are several factors which may have influenced the ability of

this study to detect changes in plasma caffeine, and results here are limited to the caffeinated

tobacco products analyzed as well as the conditions used. Under these conditions, there was no

evidence for a nicotine and caffeine interaction. Current study findings do not address the

capacity to which other caffeinated waterpipe tobacco products or any combustible caffeinated

product may deliver caffeine or interact with nicotine-related effects.

For the current study, results from subjective measures did not indicate reward-related or

pleasurable effects were enhanced in either caffeinated condition (CAFF/NIC or CAFF/LN)

relative to the nicotine-only condition (NIC). For example, the mean score for the item "Do you

like the drug effects?" was increased significantly relative to baseline at 30 minutes post product administration during NIC, and scores did not differ significantly for CAFF/NIC and CAFF/LN. Other items, such as "Do you feel a rush?", "Do you feel any drug effects?", "Lightheaded", "Sweaty, and "How high are you?", also were influenced by nicotine exposure as indexed by peak effects analysis, but these effects did not differ significantly between NIC and caffeinated tobacco product conditions. In addition, there were four subjective items that showed effects specific to the caffeinated product conditions (CAFF/LN and CAFF/NIC): DES item "Do you feel any bad drug effects?", POMS factors tension/anxiety and depression/dejection, and the PANAS scale of negative affect. Scores for the item "Do you feel any bad drug effects?" were significantly increased relative to baseline at three time points post product administration when scores were averaged across caffeine containing products (CAFF/LN, CAFF/NIC; 30, 45, and 60 minutes). While the time course analysis indicated a significant caffeine by time effect for the POMS factors tension/anxiety and depression/dejection and the PANAS scale of negative affect, for none of these items was a significant difference relative to baseline observed. While minor, these effects may have been mediated by nicotine exposure (observed during CAFF/NIC and CAFF/LN) and other product differences (e.g., additives, taste differences) that were not present in NIC or PLACEBO. Importantly, these measures indicated potentially adverse rather than positive effects associated with caffeinated tobacco products and are not likely to contribute to the reinforcing effects of these products. Overall, there were few subjective measures that significantly differed between product conditions including PLACEBO.

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In summary, many of the findings with respect to the effects of tobacco containing and placebo waterpipe preparations in the current study were similar to those reported elsewhere (Blank et al., 2011). In addition, this study revealed no evidence of caffeine exposure when waterpipe smokers smoke a waterpipe loaded with 10 grams of a caffeinated waterpipe tobacco preparation, and also no indication of enhanced positive effects produced when smoking this preparation, at least under double-blind conditions. Interestingly, anecdotal reports regarding Tangiers F-line (CAFF/NIC) contrast with the current study findings: "Tangiers caffeinated line of hookah tobacco will knock you out of your boots" (TimL, 9/19/08, Yelp.com), and "If regular Tangiers can give you a massive buzz, the F-Line brings it to a whole new level!" (Zeodynamic, 6/28/07, Hookahpro.com). Together, these results and user reports suggest that, if present, the enhanced effects of caffeinated waterpipe tobacco may be due to other non-pharmacological influences.

Potential Non-pharmacological Influences on Waterpipe Smoking Behavior

Results reported here and elsewhere (Blank et al., 2011) suggest that there is little difference in subjective response between waterpipe tobacco products that contain nicotine and those that do not. Potentially, this observation may indicate that other non-pharmacological factors and/or the "placebo effect" may be influencing user behavior and subjective response when individuals smoke non-nicotine-containing waterpipe products. Evidence from the cigarette smoking literature demonstrates that the administration of denicotinized cigarettes suppresses tobacco abstinence symptoms (Buchhalter et al., 2001; Butschky, Bailey, Henningfield, & Pickworth, 1995; Rose, Behm, Westman, & Johnson, 2000) and reduces *ad*

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libitum smoking rates (Rose, Behm, Westman, Bates, & Salley, 2003) similarly to own brand smoking. Some have suggested that non-pharmacological factors such as sensory stimulation (e.g., smell, smoke in the throat) and motor components (e.g., lighting the cigarette, moving the cigarette to mouth) can produce significant effects on smoking behavior even in the absence of nicotine (Rose, 2006; Rose, Tashkin, Ertle, Zinser, & Lafer, 1985; Westman, Behm, & Rose, 1996). These various stimuli may acquire reinforcing properties via an associative mechanism such as classical conditioning (Rose & Levin, 1991).

Besides the effects of prior conditioning, placebo effects also may be shaped by an individual's expectations about a product's drug content (e.g., nicotine, caffeine) via information from others or product packaging (see Figure 18) and expectations of the potential effects of that drug has on mood, behavior, and physiological sensations (Kirsch, 1999; Perkins et al., 2003). Expectancies that depend on contextual stimuli of instructions or salient cues are termed stimulus dependencies (Perkins, Sayette, Conklin, & Caggiula, 2003) and expectancies that refer to beliefs about the likely effects of a drug are termed response expectancies (Maddux, 1999; Martin & Sayette, 1993). Response expectancies are associated usually with consequences of drug administration, such as fear, arousal, pain, and physiological reactions. For example, cigarette response expectancies may be weight control, positive mood enhancement, and/or relief from urge or craving and waterpipe tobacco smoking response expectancies may be relaxation and/or stress reduction (Smith-Simone et al., 2008). Specifically manipulating stimulus expectancies (e.g., telling participants they are receiving drug or no drug) can activate response expectancies associated with administration of that drug or with no drug.

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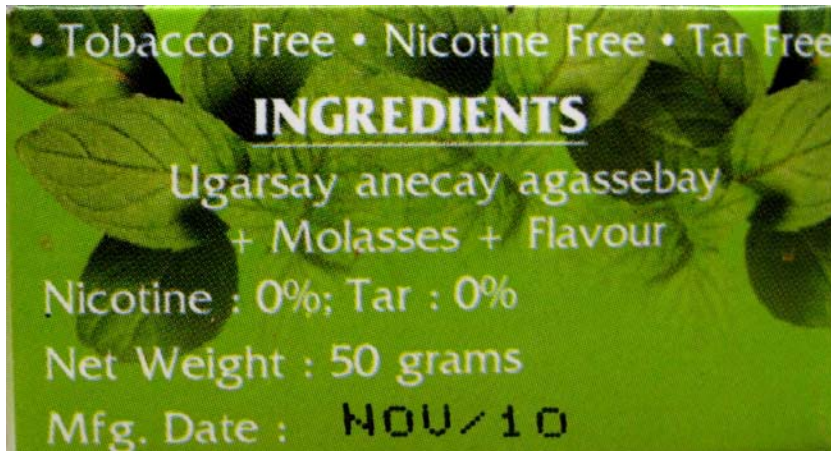
Figure 18. Labels from three waterpipe product brands: Tangiers (top), Nakha (middle), and Soex (bottom).

In the current study, many of the cues present during each session were chosen to be consistent with participant's smoking history including dim lighting, videos, comfortable chairs, waterpipe, quick-lighting charcoal, and flavored tobacco (albeit not the participants' favorite flavor). Likely, many of smoke-related sensations were similar during the current study as when participants were engaged in typical smoking behavior. Without any instructions or direct manipulation of stimulus expectancies (Perkins et al., 2003), these cues may have acted as conditioned stimuli which could have activated conditioned responses and/or stimulus and response expectancies associated with typical (nicotine-containing) waterpipe tobacco smoking. Sensory characteristics play an important role in smoking and ratings of satisfaction and some deem them "critical in mediating the immediate subjective response to smoking" (Rose et al., 2000). If these conditioned effects were activated during all study sessions, it is possible that differences between product types (i.e., particularly PLACEBO and nicotine-containing products) may have been more difficult to discern.

This study and another placebo-controlled waterpipe examination (Blank et al., 2011) demonstrated that in experienced users nicotine may be unrelated to subjective effects on many measures for waterpipe tobacco smoking at least in a single 45-minute laboratory session. Potentially, previous repeated pairings of the nicotine with waterpipe-associated cues such as taste, smell, and other stimuli may have produced a strong conditioned response in this population. Perhaps a better way to understand the effects of these different waterpipe products would be instead to manipulate systematically stimulus expectancies of certain products or in the case of current study, the drugs nicotine and caffeine.

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Future Study Design for Caffeinated Waterpipe Tobacco

Some agree that the best method to examine the specific influences of active drug and stimulus expectancies separately and together may be the balanced-placebo design (Marlatt & Rohsenow, 1981). For this two by two between-subjects manipulation, participants are randomly assigned to one of four conditions, corresponding to each combination of instruction (told drug vs. told no drug) and actual drug administration (given drug vs. given no drug). Thus, four effects are analyzed and the interaction of expectancies and pharmacological stimuli can be discriminated (Brandon, Juliano, & Copeland, 1999; Perkins et al., 2004; Juliano & Brandon, 2002).

A representative study that utilized this design examined the role of instructional set (concerning nicotine content) and actual nicotine content on measures of anxiety among 132 cigarette smokers (Juliano & Brandon, 2002). Participants were assigned to one of four conditions in a clinical laboratory setting: told nicotine/given nicotine, told nicotine/given de-nicotinized cigarette (de-nic), told de-nic/given nicotine, and told de-nic/given de-nic. Prior to cigarette administration, anxiety was induced by telling participants that they would give a short speech that would be videotaped. Manipulation checks indicated that 12% of participants reported they were deceived about the content of the cigarette, and this suspicion was distributed evenly across the four groups. Overall, the authors deemed the instruction set manipulation was effective for 74% of the total sample and analyses were restricted to these individuals except for certain cases. Results indicated that individuals who smoked nicotine cigarettes experienced greater anxiety reduction and there was no effect of instructional set. Analysis of self-reported

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urge and craving indicated that instructional set had no effects in reductions of urge for those given nicotine, but among those given de-nic, individuals who were told nicotine had greater reductions in urge compared to those who were told de-nic. Additional analyses indicated that for measures of anxiety reduction post-smoking for the full set of participants (N = 132), the impact of the instructional set manipulation was related to participants baseline expectancies (high or low) that smoking reduced negative affect. Specifically, individuals with high expectancies for negative affect reduction who were told nicotine reported higher levels of anxiety reduction, while those told no nicotine had less reduction. This interaction was less robust for those with low baseline expectancies of negative affect reduction from smoking. This finding suggested to the authors that instructional set may have greater effects for those with these stronger expectancies (Juliano & Brandon, 2002).

To address these influences, a study that manipulates stimulus expectancies as well as actual product content may be the best means to determine whether pharmacological and/or non-pharmacological factors mediate anecdotal reports concerning caffeinated waterpipe tobacco. This proposed study again would aim to examine whether the effects of caffeinated waterpipe tobacco differ to those associated with smoking typical waterpipe tobacco products (nicotine-containing). Individuals who regularly smoked waterpipe and ingested caffeine would be recruited and randomized to one of four conditions: (1) told nicotine/caffeine and smoked nicotine/caffeine, (2) told nicotine/caffeine and smoked nicotine, (3) told nicotine and smoked nicotine/caffeine, (4) told nicotine and smoked nicotine. Physiological and subjective responses would be recorded during each session. In addition to the subjective measures used in the

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current study, subjective items would also assess tobacco abstinence symptom suppression and smoking satisfaction. Also, an objective measure of behavior/performance would be included. This task would need to be sensitive to drug effects of nicotine and caffeine and could assess vigilance and/or memory. Importantly, baseline expectancies would need to be assessed by either a novel or adapted measure such as the Smoking Consequences Questionnaire (SCQ; Brandon & Baker, 1991). This scale would help identify individuals with varying levels of positive expectancies associated with smoking. These expectancies may interact with how these products induce mood changes and behavioral or subjective responses. To induce appropriate stimulus expectancies, participants would be read a prepared script concerning the product smoked during session. This script would give examples of typical side effects associated with the product assignment (i.e., potential nicotine-related effects and/or caffeine-related effects). Another important feature would be manipulation checks after product administration (i.e., "Did you believe you received nicotine and/or caffeine today?").

If the subjective measure results from the proposed study indicated that the groups who were deceived about the drug content of the products they smoked (i.e., told nicotine/caffeine and smoked nicotine or told nicotine and smoked nicotine/caffeine) did not differ from groups were given congruent instructions and drug content (i.e., told nicotine/caffeine and smoked nicotine/caffeine or told nicotine and smoked nicotine), one may be able to conclude that non-pharmacological factors have more influence than the actual product content.   If both "told nicotine/caffeine" groups reported greater stimulant-like effects than both "told nicotine" groups,

one may conclude that stimulus/response expectancies may be responsible for the anecdotal reports observed.

Limitations of the Current Study

Data from the current study must be interpreted within the context of several study limitations. These limitations include the participant population, the study setting, study design, and Type I and Type II error.

Participants were frequent users of waterpipe who smoked on average 11.4 occasions per month for on average 2.2 years, but it is possible that those with a higher use frequency (i.e., daily use; potentially more nicotine/tobacco dependent) could report different effects when using these products. If in fact higher frequency waterpipe users are more nicotine dependent, they may be more sensitive to product differences in nicotine content and could have rated the low nicotine and nicotine-free preparation with lower ratings of "like drug effects" or "rush" compared to the products with more nicotine. In addition, participants with more experience (longer waterpipe tobacco use history) may have produced a different pattern of smoke topography and thus impacted CO and or nicotine/caffeine exposure. Unfortunately, there are few studies on the various patterns of use for waterpipe tobacco smokers. One example among a group of high frequency Syrian waterpipe tobacco smokers (N=61; M =7.8 waterpipe episodes/week for on average 8.5 years) indicated during a single session this group inhaled a larger total smoke volume ($M = 79.1$ L; Maziak et al., 2009) than the current study (NIC M = 31.2 L). Many attributes of smoke topography including total smoke volume are positively correlated with nicotine and CO delivery. If experienced users inhaled more smoke, higher

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nicotine and/or higher plasma caffeine concentrations may have been observed which could have impacted cardiovascular and/or subjective effects. More smoke inhalation or more frequent puffing also could alter the temperature of the tobacco potentially producing a different exposure profile of the measured constituents: nicotine, caffeine, and CO.

Another important design feature that may have influenced smoking behavior was that individuals smoked alone, and group use of the waterpipe often is reported in the U.S. (Smith-Simone et al., 2008; Ward et al., 2007). The influence of sharing the waterpipe has not yet been assessed empirically, but one potential implication is that during sharing the IPI or the time between puffs may be shortened, and the waterpipe tobacco may be heated to higher temperatures than those observed during singleton use. Higher temperatures could have affected caffeine volatilization, but currently there is no way to determine whether this factor had any effect on caffeine exposure.

Other features of the design of this study may have influenced the ability to detect blood plasma caffeine concentration increases. For example, the lack of control over dose in this study is a limitation not easily remedied. Previous examinations of novel nicotine/tobacco products have used methods to control dose specifically such as controlled puffing regimens (Advance cigarettes, Breland et al., 2002; electronic nicotine delivery devices, Vansickel et al., 2010), gum chewing protocols (nicotine gum; Blank et al., 2008), or short administration periods (snus, nicotine lozenge; Cobb et al., 2010). A future study of waterpipe products that contain nicotine and/or caffeine could utilize a controlled puffing regimen to limit dose-related variability among participants. The head size and amount of tobacco loaded is another dose-related consideration.

Increasing the amount of the tobacco or product in the head would have increased the amount of

nicotine and caffeine available for volatilization. A larger head termed a "phunnel" is often used

to smoke the Tangiers brand of tobacco. This head is characterized by its large size and raised

center that is in contrast to a typical waterpipe head with small holes lining the bottom (see

Figure 19). To fill this head adequately, approximately 20 g of product would be necessary

compared to 10 g used in the current study. Considering the caffeine concentration per gram of

tobacco (0.5 mg), 10 mg of caffeine would be available for inhalation per "phunnel" head with a

potential increase in plasma caffeine concentration of 131 ng/ml (10*13.12 ng/ml; Zandvliet et

al., 2005).

Another important design feature was the CAFF/LN condition. An ideal comparison

condition would have been a product that contained no nicotine and only caffeine, but this

product was unavailable. With this caffeine only condition, we may have been able to determine

whether there were any specific caffeine-related effects (if there was caffeine exposure). Lastly,

blindness integrity assessment at the conclusion of each session may have been beneficial to

determine whether double-blinding was successful (Mooney, White, & Hatsukami, 2004).

Any study using inferential statistics is susceptible to rejecting the null hypothesis when it is true

(Type I error) and/or failing to reject the null when it is false (Type II: Keppel, 1991). As 231 *F*-

tests were reported for the time course analysis, some (5%; 12) of the significant results may by

chance reflect Type 1 error. However, for some of the variables that the null hypothesis was

rejected (i.e., statistically significant), a similar pattern of results was observed for the time

course and for the peak effects analysis (e.g., POMS factors tension/anxiety and

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Figure 19. Waterpipe tobacco head used in the current study (left) and the "phunnel" (right).

depression/dejection, PANAS Factor NA) or a pattern of results repeated those that were previously reported among waterpipe smokers (e.g., gradual increase in plasma nicotine; HR increase during nicotine exposure; Cobb et al., 2011; Blank et al., 2011; CO increase in response to high smoke volumes; Maziak et al., 2009). These findings suggest that Type I error did not influence the conclusions that can be drawn from this study. In addition, the use of Tukey's HSD, a conservative post-hoc test that maintains a specific alpha level across multiple comparisons (Howell, 1992) minimizes the influence of Type I error.

The Type II error rate is a function of several factors including sample size and the alpha level. The initial sample size of 40 in this study was based on previous work (Blank et al., 2007) and other power calculations that suggested that thirty participants were necessary to detect a moderate effect size (i.e., $f \geq 0.35$) with a small or moderate correlation between repeated measures (i.e., $r \geq 0.50$) with a power of 0.80 and alpha level < 0.05 (Barcikowski & Robey, 1985). The current sample size of 32 was deemed sufficient after careful inspection of individual raw plasma caffeine concentration data from the first twenty completers. During inspection, there were very few indications of a systematic increase in plasma caffeine during a session where a caffeinated product was administered. Figures 20 and 21 display the raw data of 32 study completers for each of the caffeinated product conditions (CAFF/LN; CAFF/NIC, results from one individual omitted for ease of presentation). In addition, the low dose of caffeine present in the CAFF/NIC and CAFF/LN preparations suggested that expected plasma caffeine exposure would be very low. Importantly, the current study was well-powered to detect increases in plasma nicotine concentration.
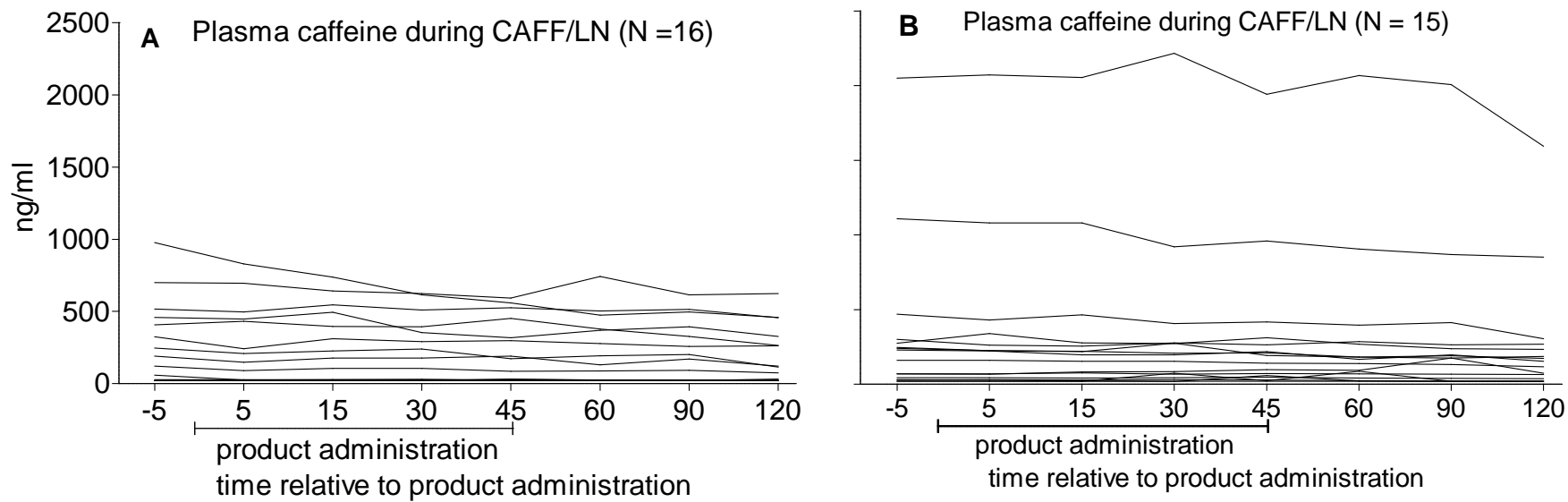
Figure 20. Raw plasma caffeine concentrations during the CAFF/LN condition (low nicotine/caffeine) from the first 16 study completers (Panel A) and the following 16 study completers (Panel B).

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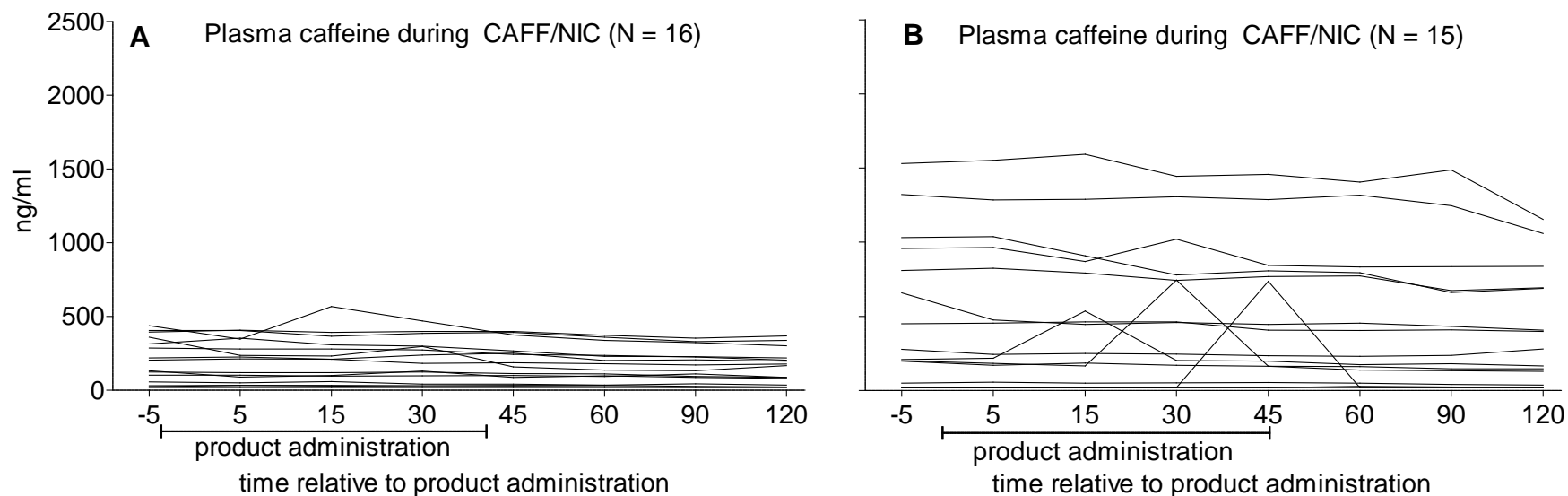
Figure 21. Raw plasma caffeine concentrations during the CAFF/NIC condition (caffeine/nicotine) from the first 16 study completers (Panel A) and the following 15 study completers (Panel B). Data from one individual who completed the study were omitted from Panel B due to extremely high levels at baseline and to allow more precise visual inspection of the other participants' results. Timecourse data for the omitted participant in minutes (min) relative to product administration are as follows: -5 min=5123 ng/ml, 5 min=5372 ng/ml, 15 min=5393 ng/ml, 30 min=5221 ng/ml, 45 min=5972 ng/ml, 60 min=5021 ng/ml, 90 min=4272 ng/ml, and 120 min=4673 ng/ml.

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Conclusions

The current study replicated many effects that have been reported elsewhere in the clinical laboratory waterpipe tobacco smoking literature (Blank et al., 2011; Maziak et al., 2009; Jacob III et al., 2011; Shafagoj et al., 2002). These effects included significant plasma nicotine exposure when products smoked contained nicotine and cardiovascular changes associated with nicotine administration. In addition, significant expired air CO exposure was observed during all conditions. Results also suggested that nicotine/caffeine content in the waterpipe tobacco preparation smoked may not induce significant differences in subjective effects under the conditions utilized here and elsewhere (Blank et al., 2011). The influence of non-pharmacological stimuli on patterns of waterpipe tobacco smoking and subjective effects is an important consideration for future examinations of this tobacco use method. Findings also indicated there was no exposure to tobacco smoke-delivered (i.e., volatilized) caffeine during this study, and thus there was no evidence for a potential nicotine and caffeine interaction when smoking these products. While these findings do not address the issue of whether caffeine can be delivered via volatilization, they did suggest that for the products examined and under the conditions explored here measurable caffeine exposure was not observed.

Importantly, tobacco dependence and toxicity capabilities are still concerns for these and other waterpipe tobacco products. While the dependence likelihood of waterpipe tobacco smoking has yet to be quantified, there is early evidence that waterpipe tobacco smokers report some difficulty quitting (Ward et al., 2005) and smoking to relieve negative affect (Auf et al., 2011). In another clinical study, significant increases in the metabolites of known carcinogens

(NNK; polycyclic aromatic hydrocarbon) were observed following a single waterpipe tobacco smoking episode (Jacob III et al., 2011). These findings as well as a recent meta-analysis of the adverse health effects associated with waterpipe tobacco smoking (Akl et al., 2010) should give cause for alarm among the public health community and support the need for more research concerning the potential for tobacco dependence from waterpipe tobacco smoking.

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Telephone Screening Form

Interviewer: **"**I would like to ask you some questions about yourself and your health status as well as your use of nicotine, alcohol, and other drugs. The purpose of these questions is to determine whether or not you are eligible to participate in either the study/studies I just described or in any of the other studies being conducted in the lab. All of your responses are confidential. You are not required to answer any question and you may stop this interview at any time. May I begin the questions?"

Document caller's response by circling either: *Yes*   *or*   *No*

If Yes: begin form. If No: thank caller for calling.

_____

How did you hear about us/our studies?   _____
Personal Information:
1. "What is your first name?"   _____

2. "What is a phone number at which you can be contacted?" _____
4. "If we call and you are not available, may we leave a message?"
                                             Circle   Yes   or   No

5. "What is your date of birth?"   _____
6. "What is your height?"   _____ (feet and inches)
7. "What is your weight?"   _____ (pounds)

8. "Did you graduate high school?"   *Circle*   *Yes*   *or*   *No*

If Yes: Skip the next question.

9. "Did you obtain your GED?":   Circle   Yes   or   No

General health status:

10. "Do you have any chronic health concerns or problems?" *Circle*   *Yes*   *or*   *No*

        If Yes: "Please describe the concern or problem":

11. "Are you under a doctor's care for a medical condition?"   *Circle   Yes   or   No*

        If Yes: "Please describe the condition":

12.   "Are you taking any prescription or over-the-counter medications?"
                                                        Circle   Yes   or   No
            If Yes: "Please identify the medication":

13. Do you have any psychiatric conditions like depression or anxiety?
                                                        Circle   Yes   or   No
            If Yes: "Please describe the condition":

14. "Have you ever been diagnosed with high or low blood pressure?"
                                                        Circle   Yes   or   No
            If Yes: "Please indicate whether it is high or low":

Cigarette use:
15. "Do you currently smoke tobacco cigarettes?"   *Circle   Yes   or   No*

If No: Skip the remainder of this section

16. "What brand of cigarettes do you smoke?"   _____

                                Circle:
                                        i)   Hard pack / Soft Pack
                                        ii)   Regular / Light / Ultra Lt
                                        iii)   Non-menthol / Menthol
                                iv)   Regular / 100s / Other

17. "How many cigarettes/day do you smoke?"   _____ *(num of cigs)*
[Note to interviewer: Please note exact number of cigarettes per day]

18. "For how long have you smoked this number?"   _____ *(mnths or yrs)*

19. "How soon after you wake up do you smoke your first cigarette?"
                                                Circle:Within 30 min.
                                                        After 30 min.
20. "Do you find it difficult to refrain from smoking in places where it
        is forbidden (e.g., at the library, at the movies)?"   *Circle   Yes   or   No*

21. "Which cigarette would you hate to give up the most?"   *Circle: 1ˢᵗ in the morning*
                                                              Any other

22. "Do you smoke more frequently during the first hours after awakening than during the rest of the day?"   *Circle   Yes   or   No*

23. "Do you smoke if you are so ill that you are in bed most of the day?"
                                                              Circle   Yes   or   No

24. "Have you ever used an electronic cigarette?"   *Circle Yes   or*
No

If Yes: "Which product?"
        _____

                "How often do you use this product?"
        _____

Waterpipe use: "The next few questions are about smoking tobacco in a waterpipe. A waterpipe is also known as a hookah or shisha. When I ask you about smoking a waterpipe, I mean <u>tobacco smoking only</u>."

25. Have you ever tried smoking tobacco in a waterpipe, even one or two puffs?
                                                              Circle Yes
or   No
If No, skip the remainder of this section.
If "Yes" continue with this section:

26. "During the past year, have you tried smoking tobacco in a waterpipe, even one or two puffs?"
                                                              Circle Yes   or   No

27. "During the past 30 days, have you tried smoking tobacco in a waterpipe, even one or two puffs?"
                                                              Circle Yes   or   No

28. "Think back over the last 6 months. On average, about how often would you say that you smoked tobacco using a waterpipe?"

(Check one)
        Less than 2 times per month?   _____
        2-5 times per month?   _____
        5-20 times per month?   _____
        More than 21 times per month?   _____

29. "Do you own a waterpipe?" *Circle Yes or No*

30. "What brand of tobacco do you prefer?"_____

31. "What flavor of tobacco do you prefer?"
1st choice   _____
2nd choice   _____

32. "Do you ever smoke a waterpipe with a group of people?" *Circle Yes or No*

33. "Do you ever smoke a waterpipe when you are by yourself?"
Circle Yes or No

34. "Do you ever drink/consume caffeinated products while smoking the waterpipe?"
Circle Yes or No

If Yes: "Which drink/product?"   _____
        "How often do you use these drinks/products while smoking the waterpipe?"
        _____

Smokeless Tobacco Use:
35. "Do you use smokeless tobacco (i.e., snuff, dip, or chew)?"
Circle Yes or No

If No: Skip the remainder of this section.

36. "What brand of smokeless tobacco do you use?   _____

37. "How many times/day do you use smokeless tobacco?"_____

38. "For how long have you used smokeless tobacco?"   _____ *(mnths or yrs)*

39. "How many cigarettes have you smoked in the past 6 months?" _____

Caffeine use:
40. "Do you ever drink beverages which contain caffeine?"*Circle Yes or No*

If No: Skip to Question 46.

41. "How many cups of instant coffee do you drink per day?"_____

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42. "How many cups of filtered coffee do you drink per day?"_____

43. "How many cups of caffeinated (not herbal) tea do you drink per day?" _____

44. "How many cups of decaffeinated (not herbal) tea do you drink per day?" _____

45. "How many caffeinated sodas/energy drinks do you drink per day (12 ounces = 1 can)?" _____

If Yes: "Which drinks?"   _____

46. "Do you eat any foods which contain caffeine such as coffee flavored ice cream or yogurt?"
                                                    Circle   Yes   or   No

If Yes: "Which foods?"   _____
       "How often do you eat these foods?"   _____

47. "Do you take NoDoz, Vivarin, or other pills to help you stay awake?"
Circle   Yes   or   No

If Yes: "How often do you take these pills?"   _____

48. "Do you use other over-the-counter medications containing caffeine, such as Excedrin?"
                                                    Circle   Yes   or   No

If Yes: "How often do you take these medications?"   _____

49. "Do you take any prescription medications containing caffeine?"
Circle   Yes   or   No

If Yes: "Which medications do you take?   _____
       "How often do you take these medications?"   _____

Interviewer: "I'd like to ask you some additional questions about your use of alcohol and other drugs."

Alcohol use:
50. "Have you ever been treated for alcohol abuse/dependence?"
Circle   Yes   or   No

If Yes: "When was your treatment completed?": _____ (mnth/year)

51. "Do you use (drink) alcoholic beverages?" *Circle Yes or No*

If No: Skip the remainder of this section.

52. "How many alcoholic drinks (by alcohol I mean beer, wine, or liquor) do you have on a typical day? _____ *(num of drinks)*

53. "How many days out of the last 30 have you used alcohol?"_____ *(num of days)*

Marijuana use:
54. Have you ever, in your lifetime, smoked marijuana or hashish?
Circle Yes or No

If No: Skip the next question.

55. "How many days out of the last 30 have you smoked marijuana?"
_____ (number of days)

--**Other drug use:**
56. "Have you used any other illegal drugs within the past month?"
Circle Yes or No

If Yes: "Please identify which drug or drugs."

For women only:
57. "Are you currently pregnant?" *Circle Yes or No*

58. "Are you currently breast-feeding a child?" *Circle Yes or No*

59. "What was the first day of your last period?" _____

Interviewer: "Thank you for responding to these questions. I need to pass on your responses to the principal investigator who will then determine whether or not you are eligible to participate in a study; someone will contact you within approximately one week if you are eligible. If you are not eligible for any of our current studies, then you will *not* be contacted."
 [If respondent does not have a phone, they can call us back in a few days]

APPENDIX B

Title. Evaluating the acute effects of caffeinated waterpipe tobacco

VCU IRB Number: HM12422

Investigators. Dr. Thomas Eissenberg

Sponsor. National Cancer Institute

This consent form may contain words that you do not understand. Please ask the study staff to explain any words that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.

Purpose of the study. The purpose of this research study is to examine how caffeinated waterpipe tobacco smoking effects you. A waterpipe is also known as a hookah, shisha, or narghile.

Description of the study and procedures. You have indicated interest in participating in a study of waterpipe users. If you agree to join the study, you will be asked questions about your general health, smoking history, and drug and alcohol use. You will need to provide a urine sample that will be tested immediately for recent use of illicit drugs (cocaine, heroin-like drugs, benzodiazepines, and methamphetamine) and also, if you are a woman, for pregnancy. To help us protect your privacy, we have obtained a Certificate of Confidentiality from the National Institutes of Health. With this Certificate, we cannot be forced to disclose information that may identify you, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. We will use the Certificate to resist any demands for information that would identify you, except as explained below.

The Certificate of Confidentiality cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

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If you use illicit drugs, or are pregnant, you cannot participate in this study. We will also ask you questions about your background, your health history, and your use of tobacco and other substances. Your responses will be confidential.

If the urine tests and your answers to our questions indicate that you fulfill the entry criteria, we will ask you to participate in four, approximately 3-hour sessions here at the Clinical Behavioral Pharmacology Laboratory located on VCU's medical campus. The four sessions will begin at approximately the same time each day, and will be separated by at least 48 hours. Before each session, we will ask you to abstain from **all** caffeine containing food and beverages and **all** tobacco products, for at least 12 hours before each session. We will also ask you to abstain from all food for 1 hour before each session. In addition, the use of any nicotine-containing products (like the gum or patch) is prohibited. We will ask you to take a simple breath and saliva test to make sure that you have complied with these restrictions. Our tests are not perfect, but they are the only measures that we can accept to make certain that you have complied with the no caffeine/no tobacco/no nicotine restrictions.

In each of the four sessions you will be asked to smoke a waterpipe in the laboratory. We want you to smoke the waterpipe as you normally would. The waterpipe that you smoke may or may not contain your preferred brand/flavor of tobacco, but will always contain a product marketed and sold for waterpipe smoking. Please note that the product that you smoke may or may not contain tobacco and/or caffeine. The waterpipe will be loaded only once, and we ask that you use it for at least 45 minutes, though you may take as many or as few puffs as you like during that period. The waterpipe will be started with a single charcoal disk, but you may add additional ½ disks as you like. All tobacco products used in this study are available to adults in the U.S. without a prescription.

At the beginning of each session, and after you provide the breath and saliva sample used to assess compliance with the no caffeine/no tobacco restrictions, a nurse will insert a thin needle into your arm that will stay there for the entire session. This needle will be used to draw blood periodically (approximately two tablespoons per sample, 8 samples per session, for a total of approximately 1/3 cup per session or 1 and 1/3 cups for the four-day study). We use this method because participants tell us that it is more comfortable than repeated "sticks" with a needle. Over the four days that you participate in this study, we will take less blood than the amount you would give in a single donation at a blood drive. In addition to taking blood and breath samples, we will also ask you to participate in other procedures that include monitoring your heart rate and blood pressure and responding to several questionnaires to measure how you feel before, during, and after you smoke the waterpipe. When you smoke the waterpipe you will notice that it is connected to a computer. The computer is measuring how you smoke (the size and number of the puffs that you take). This information allows us to understand waterpipe tobacco smoking better. You will have an opportunity to experience all of the questionnaires and see all of the equipment before your first session.

Risks and Discomforts. You may experience some discomfort during sessions when you are not using your preferred brand/flavor of waterpipe tobacco, or when you are not using any caffeine or tobacco before a session. Side effects from tobacco abstinence can include irritability, anxiety, restlessness, excessive hunger, difficulty concentrating, and sleep disturbance. Side effects from caffeine abstinence can include headache, fatigue, decreased energy/activeness, depressed mood, difficulty concentrating, and flu-like symptoms. Though uncomfortable, these feelings are not medically dangerous. Side effects from products that contain tobacco/nicotine can include sweating, lightheadedness, dizziness, nausea, and nervousness. Side effects from products that contain caffeine can include restlessness, tension, and anxiety. These effects are unlikely in individuals who use caffeine and tobacco products regularly. You may also feel some discomfort when the nurse inserts or withdraws the needle, or when blood samples are taken. Risk of bruising, bleeding, fainting or feeling lightheaded, and infection may occur. We try very hard to minimize your discomfort at these times, and the use of a trained nurse and sterile, disposable equipment enhances comfort while reducing the risk of bruising and infection. If you find any effects or data collection procedures unacceptable, you may stop your participation at any time. Medical personnel will be on call should they be needed.

Benefits. You will derive no personal benefit from this study. However, your participation will help us in the future as we try to understand the effects of different types of tobacco products.

Costs of Participation. There is no cost to you for participation except for your time. Participating in this study will take about 12 hours in the laboratory.

Payment for Participation. You will be paid for the time that you are not using tobacco prior to session and for your time in the laboratory: you will receive $75 after the first session, $75 after the second, $100 after the third session, and $100 after the fourth session. In all, you can earn $350 for successful completion of this study.

Alternatives. This is not a therapeutic study. You have the alternative not to participate.

Confidentiality. We will not tell anyone the answers that you give us; however, information from the study and the consent form signed by you may be looked at or copied for research or legal purposes by the sponsor of the research, or by Virginia Commonwealth University.

Confidentiality of your records will be maintained by keeping all data in a locked file and in a coded database. Release of this information will be withheld, consistent with the law, unless you give permission to release this information. The information obtained in this study may be published, but your identity will not be revealed.

Compensation for Injury. Virginia Commonwealth University and the VCU Health System (formerly known as the Medical College of Virginia Hospitals) have no plan for providing long-term care or compensation in the event that you suffer injury as a result of your participation in this research study. If you are injured or if you become ill as a result of your participation in this study, contact your study nurse immediately. Your study nurse will arrange for short term emergency care or referral if it is needed. Fees for such treatment may be billed to you or to appropriate third party insurance. Your health insurance company may or may not pay for treatment of injuries as a result of your participation in this study.

Pregnancy. Every effort will be made to have women enter this study on an equal basis with men. Tobacco use may be harmful to a fetus, and pregnant women may not participate in this study. If you suspect that you are pregnant, or if you are currently breast-feeding a baby, please inform the investigator now and do not participate. We will conduct a urine pregnancy test during the screening evaluation visit to ensure that pregnant women do not participate.

Voluntary Participation and Withdrawal. You do not have to participate in this study. If you choose to participate you may stop at any time without any penalty. You may also choose not to answer particular questions that are asked in this study. The investigators will answer any questions that you may have. If you choose not to participate or to discontinue your participation, this choice will in no way affect any medical care you receive now or in the future at this institution. If during the course of the study you experience adverse effects, or if you do not comply with the study restrictions, your participation may be stopped by Dr. Eissenberg without your consent. Any significant new findings that develop during the course of the research study that may affect your willingness to continue to participate will be provided to you.

Questions. You can call Dr. Eissenberg at 827-3562 for information about the research or about research-related injury.

Participants' Rights Information. If you have questions about your rights as a research participant, you may contact:

> Office for Research Subjects Protection
> Virginia Commonwealth University
> Virginia Biotechnology Research Park, BioTech One
> 800 East Leigh Street, Suite 115
> P.O. Box 980219
> Richmond, VA 23298-0219
> Telephone: 804-828-0868

If you agree to join this study, please print and sign your name below. You will receive a copy of this consent form.

Consent. I have read this consent form. I understand the information about this study. All my questions about the study and my participation in it have been answered. I freely consent to participate in this research study.

By signing this consent form I have not waived any of the legal rights which I otherwise would have as a participant in a research study.

_____
Participant's Printed Name

_____   _____
Signature of Participant   Date

_____   _____
Signature of Person Performing Consent   Date

_____
Witness's Printed Name

_____   _____
Signature of Witness   Date

_____   _____
Signature of Investigator   Date

Vita

Caroline Oates Cobb was born on June 19, 1983, in Henrico, Virginia. She is a graduate of Trinity Episcopal School in Richmond, Virginia and received her B.A. in Psychology from American University in 2005. She began the Biopsychology program at Virginia Commonwealth University in August 2007 and received her M.S. in Biopsychology in May 2009.