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COMPARING THE EFFECTS OF MENTHOL STATUS ON THE BEHAVIORAL
PHARMACOLOGY OF SMOKING REDUCED NICOTINE CONTENT
CIGARETTES

A Thesis presented

by

Danielle R. Davis

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements
for the Degree of Master of Arts
Specializing in Psychology

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Thesis Examination Committee:

Stephen T. Higgins, Advisor
Richard Rawson, Chairperson
Diann E. Gaalema, Ph.D.
John Green, Ph.D.
Cynthia J. Forehand, Ph.D., Dean of the Graduate College

Abstract

Introduction: An active area of tobacco regulatory science research focuses on examining the effects of varying the nicotine content of cigarettes as part of a potential national policy to lower their nicotine content levels to reduce addiction potential. The present study examines differences in the behavioral effects of reduced nicotine content cigarettes related to their menthol status. Menthol is the only cigarette flavoring that is still legally permissible according to Food and Drug administration regulations.

Methods: Participants were 26 current adult smokers from three populations especially vulnerable to tobacco use and addiction (economically disadvantaged women, opioid-dependent individuals, individuals with affective disorders) dichotomized as menthol (n=11) or non-menthol (n=15) smokers. Participants completed 14 experimental sessions following acute smoking abstinence ($CO \leq 50\%$ baseline level). Across sessions, participants smoked four Spectrum research cigarettes (22nd Century Group, Clarence, NY) with varying nicotine content levels (0.4mg/g, 2.4 mg/g, 5.2 mg/g, 15.8 mg/g) or their usual brand cigarette. Research cigarettes were mentholated or non-mentholated corresponding to participants usual brand. Upon completion of smoking, participants completed tasks measuring reinforcing efficacy, subjective effects, topography, and withdrawal and craving measures. Repeated Measures Analysis of Variance was used for all analyses ($p < .05$).

Results: Main effects of menthol status, as well as interactions of nicotine dose and menthol were noted across subscales of subjective effects and direct assessments of reinforcing efficacy. Usual brand mentholated cigarettes produced a profile of equal or greater relative reinforcing effects than usual brand non-mentholated cigarettes, while mentholated research cigarettes produced a profile of effects that fell below (i.e., lower relative reinforcing effects compared to usual brand or non-mentholated cigarettes) those of non-mentholated research cigarettes.

Conclusions: Mentholated research cigarettes produce a lower profile of reinforcing and subjective effects, without discernible differences in smoking topography. The potential impact of mentholation on reinforcing efficacy and subjective effects should be considered when using Spectrum research cigarettes.

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Comprehensive Literature Review

Despite a steady decline in smoking prevalence since the landmark U.S. Surgeon's General report in 1964 highlighting the deleterious health consequences of smoking, cigarette smoking remains a serious U.S. public health problem. Smoking is responsible for an estimated 480,000 premature deaths and \$300 billion dollars annually in both health and lost productivity costs (U.S. Department of Health and Human Services, 2014). To address this public health challenge, the Family Smoking Prevention and Tobacco Control Act was passed in 2009 giving the Food and Drug Administration (FDA) regulatory authority over the manufacturing and marketing of cigarettes and other tobacco products. This Act gives the FDA authority to reduce, although not eliminate, the amount of nicotine in cigarettes if deemed in the interest of protecting public health.

NICOTINE REDUCTION

There is overwhelming evidence that nicotine is the constituent in tobacco smoke that promotes chronic smoking and nicotine dependence (U.S. Department of Health and Human Services, 1988, 2012, 2014). Currently, the FDA is supporting research investigating, among other things, the impact of reducing nicotine content levels in cigarettes below a hypothesized addiction threshold (Benowitz & Henningfield, 1994). Research supported by these initiatives has suggested that acute exposure to cigarettes with reduced nicotine content decreases the reinforcing effects and other subjective effects of smoking at these lowered doses compared to cigarettes with higher nicotine contents without evidence of promoting compensatory smoking (Higgins et al., 2017). Investigations of extended exposure to cigarettes with reduced nicotine content levels

illustrate a decrease in cigarettes smoked per day (CPD) and nicotine dependence severity relative to cigarettes with a nicotine content level similar to what is in commercial cigarettes (e.g., Donny et al., 2015). This research is being used to help the FDA examine the feasibility and potential efficacy of a national policy that reduces the maximal nicotine content of cigarettes to very low levels. Importantly, as smoking rates continue to decline, certain populations, who may be especially vulnerable to smoking, show increases in smoking, higher dependence levels, and lower cessation rates (Higgins et al. 2016; Higgins & Chilcoat 2009; Hiscock et al. 2012).

MENTHOLATION IN CIGARETTES

Other constituents of cigarette tobacco that may have an impact on smoking prevalence and dependence risk are being considered by the FDA as well. One constituent that affects a large proportion of current cigarette smokers is cigarette flavoring (i.e., mentholation). In addition to giving the FDA authority to set nicotine standards, the 2009 Family Smoking Prevention and Tobacco Control Act grants FDA authority over flavorings in cigarettes including menthol (Family Smoking Prevention and Tobacco Control Act, 2009). Currently all flavorings in cigarettes other than menthol are banned. Approximately one-third of the U.S. cigarette market is made up of menthol-flavored cigarettes (Federal Trade Commission Report, 2013). Menthol cigarettes are distinct from non-menthol cigarettes due to their unique sensory effects, such as the reported ‘cooling’ that menthol provides. Individuals who smoke menthol cigarettes tend to do so exclusively and generally express preference for the sensory experience provided by the menthol cigarette (Kreslake, Wayne, Connolly 2008). As the only flavoring legally

allowable in cigarettes, it is important to consider the impact mentholation could potentially have on the acceptability of reduced nicotine content cigarettes.

In the way of background, menthol is a chemical compound derived from the oil of the peppermint and corn mint plants or can be created synthetically to provide a distinct mint-like flavor (Tobacco Product and Scientific Advisory Committee (TPSAC) Report, 2011). Menthol was first added to cigarettes in the mid 1920s, yet did not reach mainstream popularity until the mid 1930s (Tobacco Product and Scientific Advisory Committee (TPSAC) Report, 2011). Menthol flavoring is added to cigarettes in a variety of ways, most commonly by spraying an alcohol based menthol solution on the tobacco during blending or applying menthol product to the filter during cigarette filter manufacturing. Other technologies to add menthol to cigarettes include spraying an alcohol based menthol solution onto the inner foil of the packaging, inserting crushable mentholated beads in the filter, mentholated threads, or mentholated crushable microbeads that fill a filler cavity or are mixed into the filter (R.J. Reynolds, Altria Client Services, 2010). Although cigarettes marketed as non-menthol often have some level of menthol (< 0.1 mg/cigarette) in the product (Ai et al., 2016), detectable levels of menthol in cigarettes are approximately 0.6 – 1.5 mg/cigarette (Heck et al., 2010). Recent analyses of 23 non-menthol and menthol cigarette brands determined that menthol content in cigarettes advertised as menthol range widely from 2.9 – 19.6 mg/cigarette and menthol content in non-menthol cigarettes ranges from 0.0002 to 0.07mg/cigarette (Ai et al., 2016).

An estimated 39% of U.S. current smokers report regular use of mentholated cigarettes (Villanti et al., 2016). This represents an increase from 35% in 2008-2010

(Villanti et al., 2016), suggesting that while overall smoking prevalence continues to decline, menthol cigarette use does not follow that pattern. Smokers of menthol cigarettes tend to be younger, non-white, and female relative to non-menthol smokers (Giovino et al., 2015; Lawrence et al., 2010; SAMSHA, 2011; Villanti et al., 2016). There are mixed findings on dependence and smoking cessation among menthol smokers (see Hoffman & Simmons, 2011a and Frost-Pineda, Muhammad-Kah, Rimmer, & Liang, 2014 for reviews) with some literature suggesting greater nicotine dependence and lower cessation rates in menthol smokers (Ahijevych & Garrett, 2010; Fagan et al., 2015) and others suggesting no differences (Hoffman & Sommons, 2011b; Hyland, Garten, Giovino, & Cummings, 2002).

There is a literature examining the role that mentholation has on the sensory experience of smoking and how associated responses to smoking, such as craving, withdrawal, and subjective experience are affected. This literature suggests that mentholation acts as a cue or discriminative stimulus for menthol smokers and that removal or altering of that stimulus can affect the smoking experience (Rose & Behm, 2004). A recent study comparing withdrawal and craving reduction among menthol and non-menthol smokers illustrates this point. When intravenous (IV) nicotine was administered under acute abstinence to both the menthol and non-menthol smoker groups, menthol smokers reported significantly less reduction of smoking urges compared to non-menthol smokers and a trend towards less reduction of withdrawal symptoms (Devito et al., 2016). Those differences remained after controlling for potential confounding of race and gender. This blunted response to the IV nicotine in the menthol relative to non-menthol group suggests that menthol may provide a distinct stimulus

profile that at least in part may mediate other effects of smoking such as changes in craving and withdrawal as outlined above. Considered together, the literature on mentholation suggests that its potential influence on the smoking experience should be considered in any research involving menthol smokers.

MENTHOL CONTENT AND NICOTINE REDUCTION

To my knowledge, only two studies have been reported addressing the question of how smokers of non-menthol versus menthol reduced nicotine content cigarettes may differ. Pickworth and colleagues (2002) administered three cigarettes with varying nicotine yield in a double-blind, within-session study; a commercial cigarette with a yield typical of commercial cigarettes (1.1 mg), a high nicotine yield research cigarette (2.5 mg), and a low nicotine yield research cigarette (0.2 mg). It should be noted that in this study nicotine yield is used as an indicator of potential nicotine exposure level as opposed to nicotine content. Nicotine yield is machine-measured exposure to nicotine when a cigarette is smoked in a standardized way (Federal Trade Commission Report, 2000). Often smokers will engage in compensatory smoking (e.g., increasing puff volume) to obtain a greater nicotine yield than the machine-estimated value (see Scherer and Lee 2014 for a review). Thirty-six participants were assigned their preferred mentholation (i.e. menthol or non-menthol) and directed to smoke all three cigarettes over a single laboratory session. Half of the subjects were regular menthol smokers and half non-menthol smokers. Ratings of subjective experience were assessed using the Cigarette Evaluation Questionnaire (CEQ) (Westman et al., 1992) and the Duke Sensory Questionnaire (Rose & Behm, 2004). Both questionnaires were developed to understand the sensory and positive subjective effects of smoking. Number of puffs taken for each

cigarette and time to smoke each cigarette was examined as a proxy for compensatory smoking. Physiological measures of heart rate, blood pressure, and changes in carbon monoxide (CO) were collected as well. No differences by menthol status were reported for physiological measures and the commercial and high-yield cigarettes produced greater changes in physiological outcomes than the low-yield cigarette overall. There was a significant effect of mentholation on subjective effects, although it was restricted to only the high dose. At the highest nicotine yield cigarette, menthol smokers reported the cigarette to be less satisfying and provided less relief from craving compared to non-menthol smokers (Pickworth et al., 2002).

The second study (Hatsukami et al., 2013) was an initial examination of Spectrum research cigarettes (22nd Century Group), which are currently used exclusively by the FDA for purposes of examining nicotine content and are the type that is used in the present study as well. The nicotine content levels in Spectrum cigarettes are altered by genetically modifying the tobacco. Fifty-one participants smoked four puffs of three research cigarettes with different nicotine doses (nicotine content 0.4 mg/g, 5.7-5.8 mg/g, 11.4-12.8 mg/g; nicotine yields >.04 mg, 0.3 mg, 0.6 mg, respectively) over the course of a single session in double-blind, random order. Participants were assigned menthol or non-menthol cigarettes based on their usual brand preference, with approximately half of participants (47%) being menthol cigarette smokers. After smoking each cigarette, participants were given a modified version of the CEQ used by Pickworth and colleagues where subjective effects of smoking are rated on five subscales (Satisfaction, Psychological Reward, Enjoyment of Respiratory Tract Sensations, Aversion, and Craving Reduction). They also assessed a scale of Perceived Health Risk in which

participants rated the perceived disease risk of these products as well as the Multiple Choice Procedure (MCP, Griffiths, Troisi, Silverman, & Mumford, 1993), which assesses relative reinforcing effects by having participants indicate at what monetary value they would forego a pack of cigarettes for money. Physiological measures of heart rate and blood pressure were collected as well.

Overall, reducing nicotine content produced orderly, dose-dependent decreases across the battery of dependent measures. There were also significant differences in how the menthol and non-menthol smokers rated the cigarettes. Across doses, menthol smokers rated the research cigarettes as less satisfying, psychologically rewarding, and pleasing to the respiratory tract compared to non-menthol smokers. Menthol smokers also reported smaller craving reductions than non-menthol smokers. A similar finding was seen with relative reinforcing effects in the MCP, with menthol smokers choosing to switch to money at a lower monetary value indicating less reinforcing effects compared to non-menthol smokers. No interaction between menthol status and nicotine dose was reported suggesting that ratings were shifted downward among menthol compared to non-menthol smokers across the varying dose cigarettes. That differs from the results in Pickworth et al. (2002) where differences by menthol status were discerned only at the high nicotine yield cigarette.

Considered together, these two studies suggest that when nicotine exposure levels are altered, effects may differ by menthol status of the smoker, although there is some discrepancy over where in the dose-effect curve such discrepancies are discerned. Additionally, these two studies leave some gaps where further research is needed. The first is the use of a comparison of usual brand relative to research cigarette ratings. In the

Pickworth et al. (2002) study, participants smoked mentholated or non-mentholated commercial cigarettes that corresponded with whether their usual brand was mentholated or non-mentholated, but the cigarettes were not necessarily the participant's usual brand. Previous research suggests that when menthol users are switched to another menthol cigarette of similar nicotine content, subjective ratings can be lower (Strasser et al., 2013). Therefore, it is important to use the participants' usual brand, instead of a proxy, as a true baseline. In the Hatsukami et al. (2013) study, participants first smoke their usual brand cigarette, but usual brand cigarette findings were not included in the analyses. Having a usual-brand comparison could be useful to see how smoking experience of the research cigarettes compares with the participant's typical smoking experience. Finally, findings from the prior studies could be extended by examining effects across additional outcome measures. For example, examining them across well-validated measures of smoking topography, craving and withdrawal, and additional measures of relative reinforcing effects including concurrent choice (Higgins et al., 1994) and the behavioral-economic Cigarette Purchase Task (Jacobs & Bickel, 1999) could help to elucidate the range and type of outcomes on which menthol and non-menthol users vary in response to reduced nicotine cigarettes.

CURRENT STUDY

As discussed above, the overarching goal of FDA-supported research into reduced nicotine content cigarettes is to determine how individuals may respond to a policy of reduced nicotine standards. The findings from this program of research could have substantial impact on current smokers. If menthol and non-menthol smokers respond differently to reduced nicotine content cigarettes, it is important to determine as

thoroughly as possible the nature and breadth of these differences. The aim of the current investigation is to use a rigorous, within-subject research design to examine differences by menthol status across a wide range of nicotine doses (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g of Spectrum research cigarettes) as well as by usual-brand cigarettes across a relatively comprehensive battery of outcomes assessing the behavioral pharmacology of smoking.

Journal Article

Danielle R. Davis, B.S.

University of Vermont, 1 S. Prospect St., Burlington, VT 05401

Janice Bunn, Ph.D.

University of Vermont, Joseph E. Hills Agricultural Science Building, Burlington, VT
05401

Stephen T. Higgins, Ph.D.

University of Vermont, 1 S. Prospect St., Burlington, VT 05401

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Abstract

Introduction: An active area of tobacco regulatory science research focuses on examining the effects of varying the nicotine content of cigarettes as part of a potential national policy to lower their nicotine content levels to reduce addiction potential. The present study examines differences in the behavioral effects of reduced nicotine content cigarettes related to their menthol status. Menthol is the only cigarette flavoring that is still legally permissible according to Food and Drug administration regulations.

Methods: Participants were 26 current adult smokers from three populations especially vulnerable to tobacco use and addiction (economically disadvantaged women, opioid-dependent individuals, individuals with affective disorders) dichotomized as menthol (n=11) or non-menthol (n=15) smokers. Participants completed 14 experimental sessions following acute smoking abstinence ($CO \leq 50\%$ baseline level). Across sessions, participants smoked four Spectrum research cigarettes (22nd Century Group, Clarence, NY) with varying nicotine content levels (0.4mg/g, 2.4 mg/g, 5.2 mg/g, 15.8 mg/g) or their usual brand cigarette. Research cigarettes were mentholated or non-mentholated corresponding to participants usual brand. Upon completion of smoking, participants completed tasks measuring reinforcing efficacy, subjective effects, topography, and withdrawal and craving measures. Repeated Measures Analysis of Variance was used for all analyses ($p < .05$).

Results: Main effects of menthol status, as well as interactions of nicotine dose and menthol were noted across subscales of subjective effects and direct assessments of reinforcing efficacy. Usual brand mentholated cigarettes produced a profile of equal or greater relative reinforcing effects than usual brand non-mentholated cigarettes, while mentholated research cigarettes produced a profile of effects that fell below (i.e., lower relative reinforcing effects compared to usual brand or non-mentholated cigarettes) those of non-mentholated research cigarettes.

Conclusions: Mentholated research cigarettes produce a lower profile of reinforcing and subjective effects, without discernible differences in smoking topography. The potential impact of mentholation on reinforcing efficacy and subjective effects should be considered when using Spectrum research cigarettes.

Introduction

Despite a steady decline of smoking prevalence since the landmark U.S. Surgeon's General report in 1964 highlighting the deleterious health consequences of smoking, cigarette smoking remains a serious U.S. public health problem. Smoking is responsible for 480,000 deaths and \$300 billion dollars annually in both health and lost productivity costs (U.S. Department of Health and Human Services, 2014). To address this public health challenge, the Family Smoking Prevention and Tobacco Control Act was passed in 2009 giving the Food and Drug Administration (FDA) regulatory authority over the manufacturing and marketing of cigarettes and other tobacco products. This Act gives the FDA authority to reduce, although not eliminate, the amount of nicotine in cigarettes if deemed in the interest of protecting public health.

There is overwhelming evidence that nicotine is the constituent in tobacco smoke that promotes chronic smoking and nicotine dependence (U.S. Department of Health and Human Services, 1988, 2012, 2014). Currently, the FDA is supporting research investigating, among other things, the impact of reducing nicotine content levels in cigarettes below a hypothesized addiction threshold (Benowitz & Henningfield, 1994). Research supported by these initiatives has suggested that acute exposure to cigarettes with reduced nicotine content decreases the reinforcing effects and other subjective effects of smoking at these lowered doses compared to cigarettes with higher nicotine contents without evidence of promoting compensatory smoking (Higgins et al., 2017). Investigations of extended exposure to cigarettes with reduced nicotine content levels illustrate a decrease in cigarettes smoked per day (CPD) and nicotine dependence severity

relative to cigarettes with a nicotine content level similar to what is in commercial cigarettes (e.g., Donny et al., 2015). This research is being used to help the FDA examine the feasibility and potential efficacy of a national policy that reduces the maximal nicotine content of cigarettes to very low levels. Importantly, as smoking rates continue to decline, certain populations, who may be especially vulnerable to smoking, show increases in smoking, higher dependence levels, and lower cessation rates (Higgins et al. 2016; Higgins & Chilcoat 2009; Hiscock et al. 2012).

Other constituents of cigarette tobacco that may have an impact on smoking prevalence and dependence risk are being considered by the FDA as well. One constituent that affects a large proportion of current cigarette smokers is cigarette flavoring (i.e., mentholation). In addition to giving the FDA authority to set nicotine standards, the 2009 Family Smoking Prevention and Tobacco Control Act grants FDA authority over flavorings in cigarettes including menthol (Family Smoking Prevention and Tobacco Control Act, 2009). Currently all flavorings in cigarettes other than menthol are banned. Approximately one-third of the cigarette market is made up of menthol-flavored cigarettes (Federal Trade Commission Report, 2013). Menthol cigarettes are distinct from non-menthol cigarettes due to their unique sensory effects, such as the reported ‘cooling’ that menthol provides. Individuals who smoke menthol cigarettes tend to do so exclusively and generally express preference for the sensory experience provided by the menthol cigarette (Kreslake, Wayne, Connolly 2008). As the only flavoring legally allowable in cigarettes, it is important to consider the impact mentholation could potentially have on the acceptability of reduced nicotine content cigarettes.

In the way of background, menthol is a chemical compound derived from the oil of the peppermint plant and corn mint plants or can be created synthetically to provide a distinct mint-like flavor (Tobacco Product and Scientific Advisory Committee (TPSAC) Report, 2011). Although cigarettes marketed as non-menthol often have some level of menthol (< 0.1 mg/cigarette) in the product (Ai et al., 2016), detectable levels of menthol in cigarettes are approximately 0.6 – 1.5 mg/cigarette (Heck et al., 2010) and recent analyses of 23 non-menthol and menthol cigarette brands determined that menthol content in cigarettes advertised as menthol ranges widely from 2.9 – 19.6 mg/cigarette and menthol content in non-menthol cigarettes ranges from 0.0002 to 0.07mg/cigarette (Ai et al., 2016).

An estimated 39% of U.S. current smokers report regular use of mentholated cigarettes (Villanti et al., 2016). This represents an increase from 35% in 2008-2010 (Villanti et al., 2016), suggesting that while overall rates of smoking continue to decline, menthol cigarette use does not follow that pattern. Smokers of menthol cigarettes tend to be younger, non-white, and female relative to non-menthol smokers (Giovino et al., 2015; Lawrence et al., 2010; SAMSHA, 2011; Villanti et al., 2016).

There is an existing literature examining the role that mentholation has on the sensory experience of smoking and how associated responses to smoking, such as craving, withdrawal, and subjective experience are affected. The literature suggests that mentholation acts as a cue or discriminative stimulus for menthol smokers and that removal or altering of that stimulus can affect the smoking experience (Rose & Behm, 2004; Devito et al., 2016). This literature on menthol cigarettes suggests that the potential

influence of mentholation on the smoking experience should be considered in research in menthol smokers.

To our knowledge, only two studies relevant to this question have been reported. The earlier study was an examination of the sensory and physiological effects of cigarettes that varied in nicotine yield. Pickworth and colleagues (2002) administered three cigarettes with varying nicotine yield in a double-blind, within-session study; a commercial cigarette with a yield typical of commercial cigarettes (1.1 mg), a high nicotine yield research cigarette (2.5 mg), and a low nicotine yield research cigarette (0.2 mg). Thirty-six participants were assigned their preferred mentholation (i.e menthol or non-menthol) and directed to smoke all three cigarettes over a single laboratory session. Half of the subjects were menthol smokers and half non-menthol smokers. Ratings of subjective experience, measured by the Cigarette Evaluation Questionnaire (CEQ) (Westman et al., 1992) and the Duke Sensory Questionnaire (Rose & Behm, 2004), were examined. Both questionnaires were developed to understand the sensory and positive subjective effects of smoking. Number of puffs taken for each cigarette and time to smoke each cigarette was examined as a proxy for compensatory smoking. Physiological measures of heart rate, blood pressure, and changes in carbon monoxide (CO) were collected as well. No differences by menthol status were reported for physiological measures and the commercial and high-yield cigarettes produced greater changes in physiological outcomes than the low-yield cigarette overall. There was a significant effect of mentholation on subjective effects, although it was restricted to only the high dose. At the highest nicotine yield cigarette, the menthol smokers reported the cigarette

to be less satisfying and provided less relief from craving compared to non-menthol smokers (Pickworth et al., 2002).

The second study (Hatsukami et al., 2013) was an initial examination of research cigarettes called Spectrum cigarettes (22nd Century Group), which are currently used exclusively by the FDA for purposes of examining nicotine content and are the type of cigarette used in the present study as well. The nicotine content levels in Spectrum cigarettes are altered by genetically modifying the tobacco. Fifty-one participants smoked four puffs of three research cigarettes with different nicotine doses (nicotine content 0.4 mg/g, 5.7-5.8 mg/g, 11.4-12.8 mg/g; nicotine yields >.04 mg, 0.3 mg, 0.6 mg, respectively) over the course of a single session in double-blind, random order.

Participants were assigned menthol or non-mentholated cigarettes based on their usual brand preference, with approximately half of participants (47%) being menthol cigarette smokers. After smoking each cigarette, participants were given a modified version of the CEQ used by Pickworth and colleagues where subjective effects of smoking are rated on five subscales (Satisfaction, Psychological Reward, Enjoyment of Respiratory Tract Sensations, Aversion, and Craving Reduction). They also assessed Perceived Health Risk in which participants rated the perceived disease risk of these products, as well as the Multiple Choice Procedure (Griffiths, Troisi, Silverman, & Mumford, 1993), which assesses the relative reinforcing effects by having participants indicate at what monetary value they would forgo a pack of cigarettes for money. Physiological measures of heart rate and blood pressure were collected as well.

Overall, reducing nicotine content produced ordered, dose-dependent decreases across the battery of dependent measures. There were also significant differences in the

ratings of the cigarettes between the menthol and non-menthol smokers. Across doses, menthol smokers rated the experimental cigarettes as less satisfying, less psychologically rewarding, and less pleasing to the respiratory tract compared to non-menthol smokers. Menthol smokers also reported smaller craving reductions than non-menthol smokers. A similar finding was seen with relative reinforcing effects in the MCP, with menthol smokers choosing to switch to money at a lower monetary value indicating less reinforcing effects compared to non-menthol smokers. No interaction between menthol status and nicotine dose was reported suggesting that ratings were shifted downward among menthol compared to non-menthol smokers across the varying dose cigarettes. That differs from the results in Pickworth et al. (2002) where differences by menthol status were discerned only at high nicotine yield cigarette.

Considered together, these two studies suggest that when nicotine exposure levels are altered, behavioral effects may differ by menthol status, although there is some discrepancy over where in the dose-effect curve such discrepancies are discerned. Additionally, these two studies leave some gaps where future research is needed. The first is the use of a comparison of usual brand relative to research cigarette ratings, which neither prior study examined. Having a usual-brand comparison will be useful to see how smoking experience of the research cigarettes compares with the participant's typical smoking experience. Finally, findings from the two prior studies could be extended by examining effects across additional and more precise outcome measures. For example, more precise, well-validated measures of smoking topography, measures of craving and withdrawal, and additional measures of relatively reinforcing effects including concurrent choice and behavioral-economic tasks could help to elucidate the range and type of

outcomes on which menthol and non-menthol users vary in response to reduced nicotine cigarettes.

As discussed above, the overarching goal of FDA-supported research into reduced nicotine content cigarettes is to determine how individuals may respond to a policy of reduced nicotine standards. The findings from this program of research could have substantial impact on current smokers. If menthol and non-menthol smokers respond differently to reduced nicotine content cigarettes, it is important to determine as thoroughly as possible the nature and breadth of these differences. The aim of the current investigation is to use a rigorous, within-subject research design to examine differences in behavioral effects by menthol status across a wide range of nicotine content levels (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g) as well as usual-brand cigarettes across a relatively comprehensive battery of outcomes assessing the behavioral pharmacology of smoking.

Methods

Participants

Participants were 26 daily smokers from three subpopulations especially vulnerable to smoking being investigated as part of a larger multi-site study involving three sites (University of Vermont, John Hopkins University, Brown University). The subpopulations are women of reproductive age (n = 9), opioid dependent smokers (n = 11), and smokers with affective disorders (n = 6). Inclusion criteria that apply across the three subpopulations are being 18 years of age or older, smoking greater than or equal to five cigarettes per day and providing an expired breath carbon monoxide sample of greater than 8ppm to biochemically verify smoking status. Participants also provided a negative urine toxicology screen for all illicit substances, except for marijuana (THC) as

determined by a multi panel drug test that includes marijuana (THC), cocaine, barbiturates, benzodiazepines, buprenorphine, opiates, methadone, oxycodone, phencyclidine, and a breath sample at screening with a breath alcohol level (BAL) of < .01. Exclusion criteria across the three populations include intention to quit smoking within the next month, a past month quit attempt from smoking greater than three days, exclusive use of “roll your own” cigarettes, greater than nine days past month use of other tobacco products aside from traditional, combustible cigarettes (i.e. smokeless tobacco products, cigars, e-cigarettes, and related devices), currently pregnant or trying to become pregnant, currently breastfeeding, symptoms of psychosis or dementia as determined by the MINI International Neuropsychiatric Interview structured clinical interview (MINI; Sheehan et al., 1998), current suicidal ideation, and past suicide attempt (past year for those with affective disorders, past ten years for women of reproductive age and opioid maintained individuals).

Specific inclusion criteria for socioeconomically disadvantaged women of reproductive age are females between ages 18-44 with less than Associates’ degree and not currently enrolled in a degree program. Specific inclusion criteria for opioid dependent smokers are currently receiving methadone or buprenorphine for the purposes of opioid maintenance, having maintained a stable methadone or buprenorphine dose for at least the past thirty days and no more than 30% of past 30 day urine testing positive for illicit substances as determined by their maintenance provider or study staff if necessary. Specific inclusion criteria for smokers with affective disorder are current or past 12 months diagnosis of major depressive episode or disorder, dysthymic disorder, generalized anxiety disorder, post-traumatic stress disorder, obsessive compulsive

disorder, phobia or panic disorder with or without agoraphobia as determined by the MINI structured clinical interview. The local Institutional Review Board at each site approved the study prior to being conducted and each subject signed written consent.

Procedure

Participants completed fourteen 2-4-hr sessions (≥ 48 -hrs between each session). The 14 sessions were organized into three phases: Phase 1 (Sessions 1-5) an assessment of smoking topography and subjective effects; Phase 2 (Sessions 6-11) an assessment of preference between all dose pairs in a concurrent choice schedule; Phase 3 (Sessions 12-14) an assessment of preference for each of the three lower doses at a fixed relatively low response requirement (Fixed-Ratio 10) vs. the highest dose, which was available on a progressive ratio schedule. Due to technical problems, data from Phase 3 is not included in the current report.

All visits were conducted under acute abstinence ($\leq 50\%$ baseline breath CO level collected at screening) and participants were instructed to abstain from smoking 6-8-hrs prior to the visit to maintain breath CO criterion. Each experimental session was scheduled within two hours of the time that the baseline experimental visit was scheduled to keep visits at approximately the same time of day within individual participants. Upon arrival to the laboratory, participants completed brief physiological measures including breath CO, BAL, urine toxicology screen for illicit drugs, weight, heart rate, blood pressure, and urine screen for pregnancy, if applicable. Experimental sessions were rescheduled for participants who had a breath CO $> 50\%$ baseline breath CO level, a BAL $> .03\%$, or a positive drug screen for both illicit and licit opioids, aside from prescribed methadone or buprenorphine. Positive drug screens for illicit substances

excluding opioids resulted in administration of a field sobriety test. If passed, visit was continued and if failed, the visit was rescheduled. Those with a positive pregnancy test at any point during the study were withdrawn immediately. All visits occurred \geq 48-hrs apart, but within a week of each other.

In Phase 1, participants had basic physiological measures taken upon arrival at the laboratory followed by two puffs from their usual brand cigarette under staff observation to equate time since last cigarette across participants. Experimental sessions began 30-min following completion of the two puffs. During experimental sessions, participants were instructed not to eat, drink any beverages other than water, study, or use their cellular phones. Study staff regularly monitored participants during experimental sessions. During the 30-min wait period, participants completed two assessments; the Minnesota Nicotine Withdrawal Scale (MNWS; Hughes & Hatsukami, 1986) and the Questionnaire of Smoking Urges (QSU-Brief; Cox et al., 2001).

Participants smoked two cigarettes during each visit. Each cigarette was smoked using a CReSS (Clinical Research Support System) Desktop smoking topography device (Borgwaldt KC, Richmond, VA). Cigarettes were smoked through a plastic holder that is attached to two air filled tubes, which lead to a pressure transducer (see Figure 1). The device measures and records a number of smoking topography measures; (1) total number of puffs, (2) inter-puff interval (s), (3) puff volume (mL), (4) puff duration (s), and (5) maximum puff velocity (mL/s).

In Session 1 (Baseline session) participants smoked their usual brand cigarette. Across the four subsequent experimental sessions in Phase 1 (Sessions 2-5), participants smoked one of four research cigarettes (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g) ad

libitum. Order of smoking the research cigarettes was randomized across sessions and participants and was double blind. Research cigarettes were referred to by arbitrary letter code and were identical in physical appearance. Participants were instructed to smoke the cigarette as they normally would outside of the laboratory.

Approximately two minutes after extinguishing the first cigarette in Sessions 2-5, participants lit a second cigarette of the same dose and smoked it in the controlled manner described below. This second cigarette was designed to introduce participants to the controlled puffing procedures that were to be used in later phases of the study. Participants lit the cigarette without inhaling, inserted the cigarette into the cigarette holder filter, then proceeded to begin inhaling until a 60 mL volume of smoke has been inhaled which was displayed visually on the computer screen by a counter that increased as puff volume increased; a second counter immediately next to the running counter showed the goal volume of 60 mL. Participants were instructed to hold the inhaled puff in their lungs for 5 s with a timer counting down the duration displayed on a running counter. Following initiation of a puff, a 30-sec period began to ensure at least that amount of time between initiation of each puff with the duration again displayed as a running counter on the computer screen. Participants were instructed to not take the next puff until all time on the counter had elapsed. Participants followed this regimen until the cigarette was smoked down to just above the filter.

Upon completion of the second cigarette, participants completed the modified CEQ (mCEQ; Cappelleri et al., 2007; Westman et al., 1992) and the Cigarette Purchase Task (CPT; Jacobs & Bickel, 1999; MacKillop et al., 2008). The mCEQ consists of 12 questions on five subscales: Smoking Satisfaction, Enjoyment of Respiratory Tract

Sensations, Aversion, Craving Reduction, and Psychological Reward. Participants answered each question using a Likert scale from zero to seven with an answer of zero indicating “not at all” and an answer of seven indicating “extremely”. The CPT uses an escalating series of prices to examine the relative reinforcing efficacy of cigarettes by assessing estimated cigarette consumption at varying levels of economic constraint in a 24-hr period when there is no access to any other nicotine products. In the current study, the CPT assesses consumption of cigarettes using a 17 point scale evaluating how much one would smoke when the cost is \$0.00/cigarette increasing until cost of the cigarette is \$5.00/cigarette. The CPT generates an individual demand curve using five indices: (a) Intensity (cigarette smoking when unconstrained by cost), (b) O_{\max} (maximum amount of money one is willing to spend on smoking per day), (c) P_{\max} (price at which demand decreases proportional to price increasing price) (d) Breakpoint (price at which one would quit smoking rather than incur the cost), and, (e) Elasticity of Demand (overall sensitivity of smoking rate to price). Additionally, breath CO to measure CO boost, as well as withdrawal and craving as measured by the MNWS and QSU-brief were measured every 15 minutes in the hour following completion of smoking the second cigarette. To measure CO boost, pre-cigarette CO was subtracted from CO from each value measured at each of the four time points after smoking the cigarette.

Phase 2 consisted of six 4-hr sessions. Upon arrival at the laboratory, participants followed procedures identical to Phase 1 sessions. Experimental sessions began 30 minutes following completion of the two cigarette puffs that equate time since last smoking across participants. During the 30-min wait period, participants again completed the MNWS and the QSU-brief. After 30-min elapsed, participants completed a 3-hr

concurrent choice task. Upon initiation of the task participants were presented with two different packs of cigarettes, each with a different letter code. Across the six sessions, participants experienced all available dose pairs (15.8mg/g v. 0.4 mg/g, 15.8mg/g v. 2.4 mg/g, 15.8mg/g v. 5.2 mg/g, 5.2 mg/g v. 2.4 mg/g, 5.2 mg/g v. 0.4 mg/g, 2.4 mg/g v. 0.4 mg/g) under double-blind conditions with each dose identified by letter code. Order of dose pairings was randomized across participants and letter codes corresponded to the letter codes in Phase 1. Participants were instructed to attend to the notes they had made about each research cigarette to inform their choices during the concurrent task. In the chamber participants faced a computer screen displaying two 1.25-inch squares. Each square contained a letter code indicating the two research cigarettes available that session. Participants were instructed to smoke as much or as little as they want.

Participants indicated a desire to smoke by using a computer mouse to complete 10 clicks on the letter code of the cigarette they wished to smoke. After completion of the 10 clicks, a 3-min counter appeared on the screen during which the participant could take two puffs from the selected cigarette in the controlled manner that they had practiced in Phase 1. Once two puffs were completed, the cigarette was extinguished and the butt placed in a disposal container. Each smoking choice during a session involved participants lighting a new cigarette. This controlled puffing procedure is used to control for any between or within subject differences in smoking topography and to assure that all puffs are taken from the same part of the cigarette. At completion of the 3-hr session, participants again complete the MNWS and the QSU-brief.

Study Product

Study product was Spectrum investigational research cigarettes manufactured by 22nd Century Group (Clarence, NY). Four nicotine dose conditions were investigated using research cigarettes defined by a nicotine content that was an average across menthol and non-menthol products (assignment of a menthol or non-menthol product was based on a participant's reported usual brand and self-reported preference of menthol or non-menthol product during the duration of the study): 15.8, 5.2, 2.4, and 0.4 mg/g. For the 15.8 mg/g dose, the average nicotine content for the menthol product was 15.46 mg/g and the average nicotine content for non-menthol product was 16.21 mg/g. For the 5.2 mg/g dose, the average nicotine content for the menthol product was 5.22 mg/g and the nicotine content for average non-menthol product was 5.12 mg/g. For the 2.4 mg/g dose, the nicotine content for the average menthol product was 2.38 mg/g and the nicotine content for average non-menthol product was 2.32 mg/g. Finally for the 0.4 mg/g dose, the average nicotine content for the menthol product was 0.39 mg/g and the average nicotine content for non-menthol product was 0.38 mg/g. Hereafter, doses will be referred to by the nicotine content averaged across menthol and non-menthol product: 15.8, 5.2, 2.4, and 0.4 mg/g. The highest dose (15.8 mg/g) is relatively similar to nicotine content in current commercial cigarettes and the lowest dose (0.4mg/g) is below the proposed threshold of nicotine dependence (Benowitz & Henningfield, 1994). As reported by the Center for Disease and Control, average menthol content of the mentholated research cigarettes ranges from 4.97 mg/cigarette to 7.72 mg/cigarette, with an average menthol content of 5.98 mg/cigarette at the 0.4mg/g dose, 4.97 mg/cigarette at the 2.4 mg/g dose, 6.15 mg/cigarette at the 5.2 mg/g dose, and 7.13 mg/cigarette at the

15.8 dose (Richter et al., 2016). These menthol contents are similar to commercial mentholated cigarettes (Ai et al., 2016). These cigarettes also differed in the content or yield of minor alkaloids and nitrosamines and in the application of casings, including sugars (which were higher in the cigarettes with 15.8 mg/g than in the reduced-nicotine cigarettes in order to balance the ratio of nicotine to sugar) (Donny et al., 2015).

Statistical Methods

Analysis of differences between menthol and non-menthol in demographic variables and smoking characteristics were conducted using independent samples t-tests for continuous variables and Pearson's chi-square test for categorical variables. For any categorical variables with categories involving less than five subjects, Fischer's Exact Tests were conducted.

Differences in mCEQ, CPT, and smoking topography were determined using repeated analysis measures of variance, with menthol status (use of non-menthol versus menthol cigarettes) as the between-subjects factor and nicotine dose (0.4, 2.4, 5.2, 15.8 mg/g, Usual Brand) as the within-subject factor. Analyses of MNWS, QSU, and CO boost used a similar approach, however, time (pre- and post-cigarette within each session) was added as an additional within-subject factor and nicotine dose will only include research cigarette doses (0.4, 2.4, 5.2, 15.8 mg/g) and not Usual Brand. Significant menthol, time, and dose effects and interactions were followed by post-hoc tests to fully explain the nature of the differences. The CPT consist of five indices; Intensity, Breakpoint, Pmax, Omax, and Elasticity. Breakpoint, Pmax, Omax, and Elasticity were log transformed and the indice Intensity was square root transformed to meet normality assumptions. Differences between pairings in all the six dose pairings in

the concurrent choice task were also similarly examined using repeated measures analysis of variance with menthol status as the between-subjects factor and nicotine dose pair as the within-subjects factors. Post-hoc testing followed to determine which specific dose pairs evoke the greatest differences by menthol status with the higher dose chosen at a statistically significantly higher proportion than chance. Additionally, we examined any differences in smoking bouts (i.e. number of puffs taken during Phase 2 choice procedures) by menthol and non-menthol groups. Significance for all tests was set at $p < .05$ (two tailed).

Primary Outcomes

We examined differences by menthol status and interactions of menthol status and nicotine dose in the dependent measures discussed above. More specifically, differences in the mCEQ, the CPT, the MNWS, QSU-brief, smoking topography indices, and CO boost were examined from Phase 1. From Phase 2, differences in proportion of choices allocated to the higher dose across the 6-possible dose pairs were examined, as well as overall number of smoking bouts per session.

Results

Participant Characteristics

Among the 26 participants, 42% ($n = 11$) used menthol cigarettes as their usual brand and 58% ($n = 15$) used non-menthol cigarettes. Menthol and non-menthol smokers were mainly female smokers in their mid thirties with the majority having a high school education or less. Menthol smokers were significantly more likely to be non-white than non-menthol smokers (Table 1).

mCEQ

As hypothesized, ratings of menthol cigarettes fell below those of the non-menthol cigarettes in four of the five mCEQ subscales (Figure 1) with menthol smokers rating the cigarettes lower than non-menthol smokers. On the Psychological Reward subscale, there was a main effect of menthol status ($F(1,24) = 4.74, p < .05$) and an interaction of menthol and nicotine dose ($F(4,96) = 2.90, p < .05$) with menthol ratings overlapping with non-menthol in the usual brand cigarette comparison, but falling below non-menthol ratings across each of the research cigarette doses. In contrast to the pattern seen with menthol cigarettes, ratings of the non-menthol cigarettes did not change from usual-brand levels across the differing research cigarette doses. Similar interactions of menthol status nicotine dose were seen with the subscales Smoking Satisfaction ($F(4,96) = 2.83, p < .05$) and Enjoyment of Respiratory Tract Sensations ($F(4,96) = 2.78, p < .05$). Post-hoc testing across the three subscales with menthol by nicotine dose interactions, showed significantly lower ratings of the research cigarettes compared to usual brand among menthol ($ps < .05$) but not non-menthol smokers. Menthol status produced a main effect on the Craving Reduction subscale with menthol status ratings falling below non-menthol ratings across all dose comparisons ($F(1,24) = 14.24, p < .001$). No main effect of menthol or interaction of menthol status and nicotine dose were observed on the Aversion subscale. As expected, nicotine dose produced a main effect across all five mCEQ subscales of the ($Fs(4,96) \geq 2.79, ps \leq .01$).

CPT

There were no main effects of menthol status on the five CPT indices, but there were three significant interactions of menthol status and nicotine dose on Breakpoint,

Elasticity, and Omax (Figure 2, Panels A, B, and C respectively), and two trending in the same direction, Pmax and Intensity (Panels D and E respectively). Regarding the three indices with significant interactions, ratings of the usual brand menthol cigarette either overlapped with or indicated greater value than the non-menthol cigarette. Thereafter, changes in the direction of lower value or reinforcing efficacy relative to usual brand was seen across the menthol research cigarettes whereas scores of the non-menthol research cigarettes changed little relative to usual brand ($F(4,88) = 3.52, p < .05$). Post-hoc testing revealed menthol smokers reported lower values for the research cigarettes compared to usual brand ($p < .05$) while non-menthol cigarette smokers did not. Similar patterns were seen with the two indices with non-significant trends towards an interaction ($F(4,88) < 2.24, p = .07$). There was a significant main effect of nicotine dose across four of the five CPT indices (Breakpoint, Elasticity, Omax, and Pmax) ($F(4,88) \geq 3.54, p < .01$).

Direct Testing of Reductions in Addiction Potential

Direct tests of preference across dose pairs in the concurrent-choice arrangement were of interest for assessing differences in the relative reinforcing effects of the research cigarettes by menthol status. There was no main effect of menthol status nor a menthol dose interaction. There was a significant main effect of nicotine dose ($F(5,110) = 2.37, p < .05$), with participants preferring the higher of the doses. Post hoc testing revealed that those differences were attributable to three dose pairs (0.4 v 15.8, 2.4 v 15.8, 2.4 v 5.2) (Figure 3, Panel A). Exploratory analyses of those same dose pairs by menthol status indicated that the preference for the higher over the lower dose was significant ($p < .05$) among the non-menthol, but not the menthol smokers (Figure 3, Panel B).

An analysis of the average number of total puffs across dose pairs was examined by menthol status to determine any differences in overall smoking levels. Mean number of choices to smoke in the menthol group were somewhat lower than choices of non-menthol (9.50 ± 6.76 versus 16.37 ± 13.37) but that difference was not significant ($p = .11$).

MNWS and QSU-brief Ratings

Nicotine withdrawal and craving were of interest to determine if there were differences by menthol status in the amount of relief produced by smoking. Craving and withdrawal ratings were not collected for usual-brand cigarettes, thus analyses are restricted to the four research cigarettes.

Regarding the MNWS total score and desire to smoke item, there was no main effect of menthol status, however there was a main effect of time on both MNWS Total score ($F(4, 96) = 11.39, p < .001$), and MNWS desire to smoke item ($F(4,94) = 29.41, p < .01$) suggesting ratings of withdrawal and craving immediately decreased relative to baseline (pre-smoking) and increased as time elapsed post-smoking (Figure 4). There was a significant interaction of menthol status and time with reductions in withdrawal relative to baseline (pre-smoking) ratings being discernible among the non-menthol smokers but not the menthol smokers (MNWS Total score: ($F(4,96) \cong 5.39, p < .001$; MNWS desire to smoke item: ($F(4,96) \cong 5.36, p < .05$). There was no main effect of nicotine dose on the MNWS, nor interactions of dose with menthol or time.

There was no main effect of menthol status on Factors 1 or 2 of the QSU-brief. There was a main effect of time on Factors 1 ($F(4,95) = 33.08, p < .001$) and 2 ($F(4,96) = 28.26, p < .001$), but no significant interactions of menthol status and time. There were no

main effects of nicotine dose nor significant interactions of dose, time, and menthol status on either QSU factor. There was a nonsignificant trend towards an interaction of menthol, time, and dose for both Factors 1 ($F(12,287) = 1.77, p = .05$) and 2 ($F(12,287) = 1.74, p = .06$) corresponding to the highest research dose cigarette producing larger reductions in craving at the 15- and 30-minute assessments among non menthol compared to menthol smokers (data not shown).

Smoking Topography and CO Boost

Smoking topography and CO boost were of interest due to potential concerns of low nicotine content cigarettes increasing compensatory smoking (i.e. larger puff volume, longer puff duration) and the possibility of differences in this behavior by menthol status. Smoking topography was examined across usual brand cigarettes and the research cigarettes, while CO boost was restricted to only the research cigarettes.

Across the five indices of smoking topography, there were no main effects of menthol or menthol by nicotine dose interactions, however there was a non-significant trend towards a main effect of menthol status for Mean Puff Volume ($F(1,16) = 4.32, p = .05$) corresponding to a lower puff volume among menthol smokers relative to non-menthol smokers. There was a main effect of nicotine dose on Inter-puff Interval ($F(4,60) = 2.57, p < .05$), but no other main effects of nicotine dose across the remaining indices (Mean Puff Volume, Puff Duration, Number of Puff, Maximum Flow Rate) (data not shown).

In regards to CO boost following acute exposure, there was no significant effect of menthol status, dose, or menthol by dose interactions. There was a significant main effect of time ($F(3,288) = 8.92, p < .001$) with CO levels decreasing across time (data not

shown). Overall, these results show no evidence of compensatory smoking by menthol status or dose.

Effect of Race on Menthol

It is important to note that although menthol and non-menthol smokers differed significantly by race, we were unable to control for race in the analyses due to the homogenous non-menthol sample (i.e. all identifying as Caucasian). To explore the possibility that differences in the mCEQ, CPT, and concurrent choice testing (Phase 2 dose comparisons) may have been an artifact of including racial minorities in the menthol but not the non-menthol conditions, we plotted results restricting the data to on participants who identified as Caucasian (Figure 5). Patterns seen in the complete data persisted in the sample restrict to Caucasian only, suggesting that the menthol status differences were not attributable to racial differences between the menthol and non-menthol conditions.

Conclusions

Overall, the results from this within-subjects study of acute exposure to varying nicotine content cigarette suggests that menthol smokers respond differently than non-menthol smokers and those differences follow two distinct patterns. The first pattern is a reduced acceptability of the research cigarettes in menthol compared to non-menthol smokers. This lowered response to the research cigarettes across doses among the menthol relative to non-menthol smokers is seen across measures of positive subjective effects, withdrawal and craving ratings, and reinforcing efficacy. The findings from both the subjective ratings and reinforcing efficacy are consistent with the results reported by Hatsukami et al. (2013) where menthol smokers showed less pleasurable subjective and

reinforcing effects of research cigarettes compared to non-menthol smokers across multiple doses of Spectrum research cigarettes. The present results also extended those results by demonstrating that menthol smokers also report reduced relief from nicotine withdrawal and desire to smoke compared to non-menthol smokers.

A second pattern seen in the present results is that menthol smokers compared to non-menthol rate the research cigarettes considerably less satisfying and reinforcing than their usual brand cigarette whereas non-menthol smokers did that to a lesser extent. This difference between menthol and non-menthol smokers was observed on the CPT and the mCEQ measures. To our knowledge, comparisons of usual brand and research cigarettes by menthol status have not been reported previously.

There are several possible explanations for why these differences by menthol status may occur. One possibility is that they are attributable to participant self-selection into the menthol and non-menthol categories. While we cannot rule out that possibility regarding participant characteristics not assessed in this study, among those characteristics that were compared between menthol and non-menthol smokers, only race differed significantly. We could not control for race statistically but when results were examined among only Caucasians, similar patterns of menthol versus non-menthol differences remained discernible (See Results and Figure 5), rendering confounding by race as an inadequate account of the results.

Another potential explanation is that the menthol content differs between commercially marketed cigarettes and the Spectrum research cigarettes. However, the most recent analyses of commercially marketed and Spectrum cigarettes (Ai et al., 2016 – currently marketed cigarettes; Richter et al., 2016 – Spectrum cigarettes) suggest that

Spectrum research cigarettes fall well within the middle of the range of menthol content levels (See Ai et al., 2016 for most up to date analyses). In the current study, average menthol content of usual brand cigarettes among menthol smokers was 4.99 (\pm .194) mg/cigarette and the Spectrum cigarettes used in the current study had an average menthol content of 6.25 (\pm .472) mg/cigarette. Although menthol content of usual brand was slightly lower than Spectrum cigarettes, both of these values are well within range of “normal” menthol content reported by Ai et al (2016). None of the menthol smokers in our sample for whom we had data had menthol contents that were outliers (Range: 3.84 – 5.30), although menthol content of usual brand in the current study was only available for 8/11 (73%) of the menthol smokers.

A final possible explanation for the findings could be greater unwillingness to switch products among menthol compared to non-menthol smokers. Although data examining this is sparse, Strasser et al. (2013) showed decreases in satisfaction among menthol smokers when they were switched from their usual brand to a different commercial mentholated product (Camel Crush). These results in combination with findings from the current study suggest that this lowered rating of the research cigarette relative to usual brand may not be specific to Spectrum cigarettes, but may be a characteristic of menthol smokers. More research comparing brand switching between menthol and non-menthol smokers is needed to support this conclusion.

The present study has several limitations that merit mention. One that was discussed above is the inability to control for racial differences in the sample, although excluding minorities from the analyses did not appear to alter the pattern of results to any meaningful extent. Another limitation is the relatively small sample size and one that was

restricted to populations who are especially vulnerable to smoking. However, the results were sufficiently robust to discern statistically significant results across multiple dependent measures and the results were consistent with prior studies using other populations. These limitations notwithstanding, the present findings suggest that mentholated Spectrum research cigarettes produce less pleasurable subjective effects, less relief from withdrawal and craving, and lower reinforcing efficacy among smokers whose usual brand is mentholated compared to non-mentholated. Further research will be necessary to determine why this difference is occurring, but researchers involved in tobacco regulatory science should be aware of this replicable observation and should consider its implications when conducting research with menthol smokers. Does this difference occur because Spectrum cigarettes are overall less palatable to menthol smokers due to some limitation of the research cigarette or is it because menthol smokers are less likely to be amenable to switching brands more generally? These are important questions to resolve that have potentially important implications for tobacco regulatory research.

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Table 1. Demographics

	Menthol (n = 11)	Non-Menthol (n = 15)	<i>p</i> value
Age (M ± SD)	35.73 ± 12.06	37.4 ± 10.12	.7
Gender (% Female)	72.7%	80%	.67
Race (% White)	55%	100%	.03
Education (%)			1.0
Some High School	27.3%	20%	
High School	45.5%	40%	
Grad./Equivalent			
Some College/2-Yr	27.3%	33.3%	
Degree			
College Graduate/4-Yr	0%	6.7%	
Degree			
Marital Status (%)			.13
Never Married	90.9%	46.7%	
Remarried	0%	13.3%	
Separated	0%	20.0%	
Divorced	9.1%	6.7%	
Widowed	0%	13.3%	
Cigarettes per Day (M ± SD)	16.46 ± 7.78	18.27 ± 11.8	.66
Nicotine Yield (M ± SD)	1.25 ± 0.21	1.19 ± 0.27	.59
Cigarette Type (%)			.4
Full Flavor	90.9%	78.6%	
Light	9.1%	21.4%	
Age Started Smoking Regularly (M ± SD)	15 ± 2.05	15.47 ± 2.13	.58
Fagerström Test for Nicotine Dependence (M ± SD)	4.63 ± 2.24	5.53 ± 2.97	.41

Note. Values in the table are reported as means ± standard deviations unless otherwise noted. Nicotine yield values come from the Federal Trade Commission's Tar, Nicotine, and Carbon Monoxide Report from 1999-2005. Data on cigarette type and yield was not available for 1 participant.

Figure Legends

Figure 1. Panels A-E: Mean \pm SEM for Modified Cigarette Evaluation Scale for menthol and non-menthol across usual brand (UB) and the four research cigarettes (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g). A main effect of nicotine dose was seen across subscales, a main effect of menthol status was seen at the Craving Reduction subscale (Panel D), and an nicotine dose menthol interaction effect was seen at Smoking Satisfaction (Panel A), Psychological Reward (Panel B), and Enjoyment of Respiratory Tract Sensations (Panel C). An asterisk (*) indicates a significant effect of group at doses in subscales with an interaction.

Figure 2. Panels A-E: Mean \pm SEM for the Cigarette Purchase Task for menthol and non-menthol across usual brand (UB) and the four research cigarettes (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g). Panel A: *Breakpoint* (estimated price at which participants would quit smoking rather than incur its costs). Panel B: *Elasticity* (estimated overall sensitivity of demand to price increases). Panel C: *Omax* (estimated maximal expenditure participants were willing to incur for smoking in one day). Panel D: *Pmax* (estimated price where demand begins to decrease proportional to price increases). Panel E: *Intensity* (estimated consumption levels across prices ranging from \$0 to \$40/cigarette). A main effect of nicotine dose was seen for four of the five indices; Breakpoint (Panel A), Elasticity (Panel B), Pmax (Panel C), Omax (Panel D) and a nicotine dose menthol interaction was seen at Breakpoint, Omax, Pmax. Breakpoint (Panel A), Elasticity (Panel B), Omax (Panel C), and Pmax (Panel D) are all log transformed and Intensity (Panel E) is square root transformed. No significant effect of group was seen at doses in indices with an interaction effect.

Figure 3. Mean proportion of choices for three nicotine dose pairs across concurrent choice sessions. Data points are mean across participants by menthol status and error bars represent \pm SEM. The varying two-dose comparisons are shown on the x-axis with mean proportion of choice allocated to each shown on the y-axis. Panel A: Proportion of choices across dose pairs across all subjects. Panel B: Proportion of choices across dose pairs by menthol and non-menthol smokers. Asterisks (*) indicate that the higher dose was chosen at significantly greater than chance level.

Figure 4. Mean \pm SEM for the Minnesota Nicotine Withdrawal Scale Total Score and restricted to one item; Desire for menthol and non-menthol pre smoking and across time points post smoking (15, 30, 45, 60 minutes) and across four research cigarettes (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g). Data points are mean across participants by menthol status and error bars represent \pm SEM. Panel A: MNWS Total Score across menthol subjects. Panel B: MNWS Total Score across non-menthol subjects. Panel C: MNWS Desire Item across menthol subjects. Panel D: MNWS Desire Item across non-menthol subjects.

Figure 5. Comparison of menthol data including all subjects and restricted to include only white smokers. Panel A: Mean \pm SEM for the subscale Smoking Satisfaction on the Modified Cigarette Evaluation Scale for menthol and non-menthol across usual brand (UB) and the four research cigarettes (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g) including all menthol subjects.

Panel B: Mean \pm SEM for the subscale Smoking Satisfaction on the Modified Cigarette Evaluation Scale for menthol and non-menthol across usual brand and the four research cigarettes (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g) including only white menthol subjects.

Panel C: Mean \pm SEM for the Cigarette Purchase Task indice Breakpoint for menthol and non-menthol across usual brand and the four research cigarettes (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g) including all menthol subjects.

Panel D: Mean \pm SEM for the Cigarette Purchase Task indice Breakpoint for menthol and non-menthol across usual brand and the four research cigarettes (0.4mg/g, 2.4mg/g, 5.2mg/g, 15.8mg/g) including only white menthol subjects.

Panel E: Mean proportion of choices by menthol status allocated to the different nicotine dose cigarettes across three 3-hour two-dose concurrent choice sessions included all menthol subjects. Data points are mean across participants by menthol status and error bars represent \pm SEM.

Panel F: Mean proportion of choices by menthol status allocated to the different nicotine dose cigarettes across three 3-hour two-dose concurrent choice sessions included only white menthol subjects. Data points are mean across participants by menthol status and error bars represent \pm SEM.

Figure 1. mCEQ Subscales by Menthol Status Across Dose

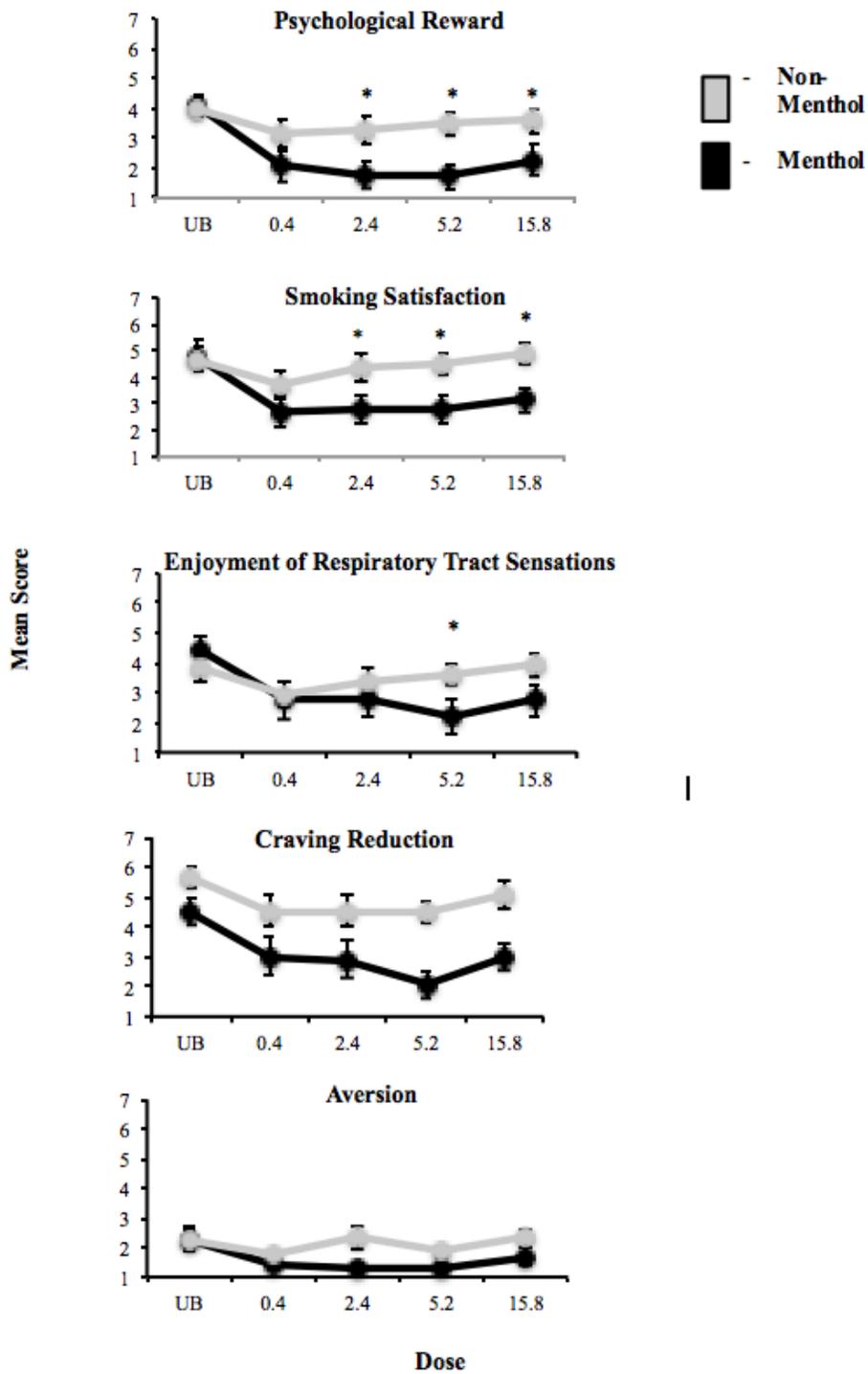


Figure 2. CPT Indices by Menthol Status Across Dose

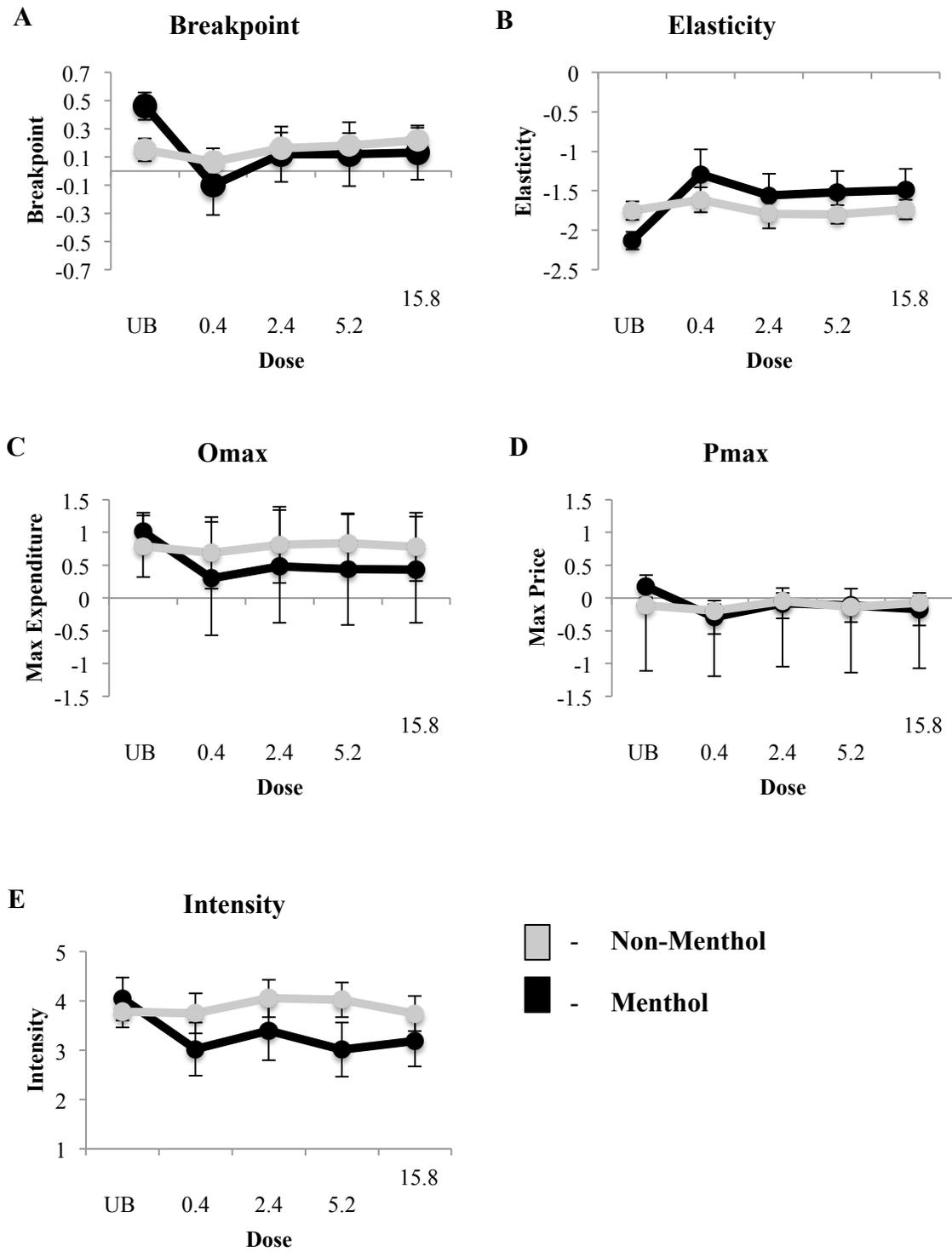
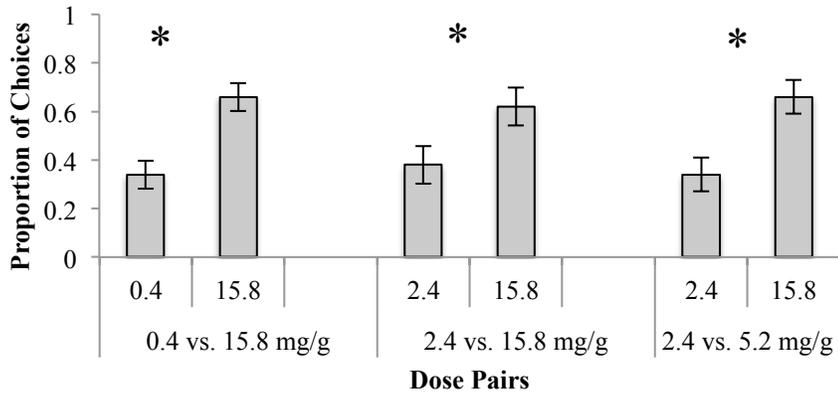


Figure 3. Concurrent Choice Test Across Dose Pairs

A

Concurrent Choice Testing



B

Concurrent Choice Testing - Menthol & Non-menthol

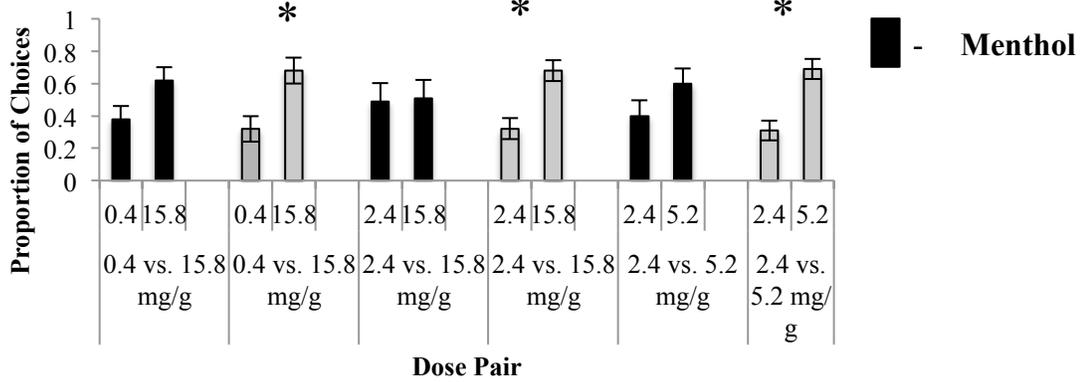


Figure 4. MNWS by Menthol Status Across Dose

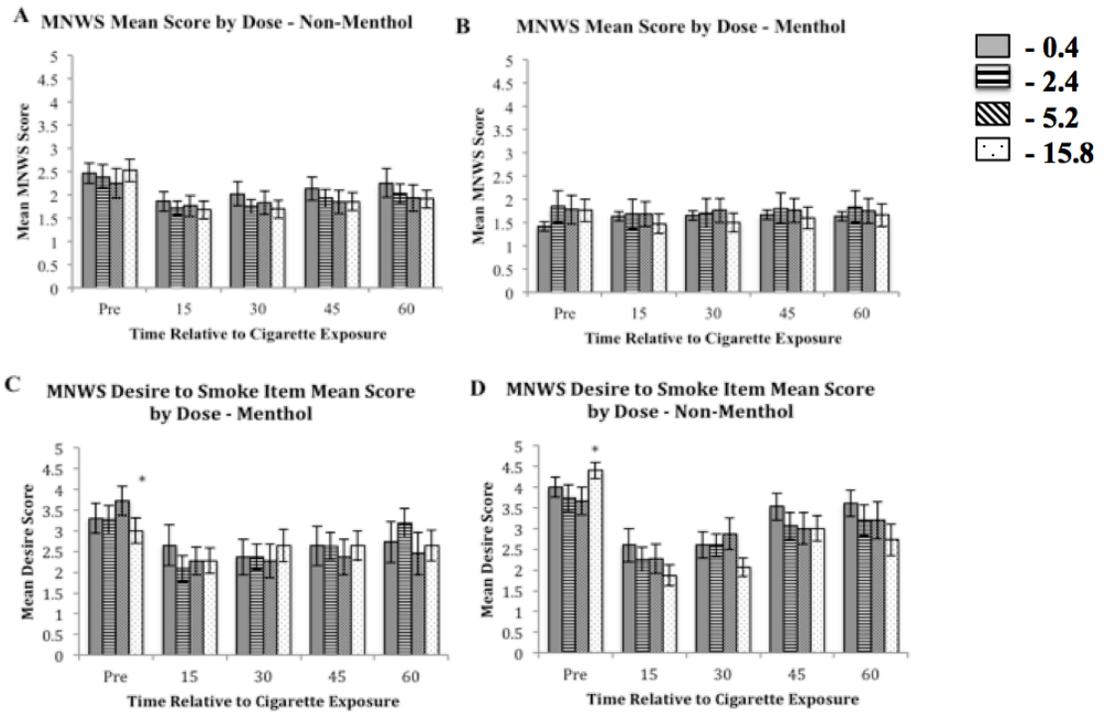
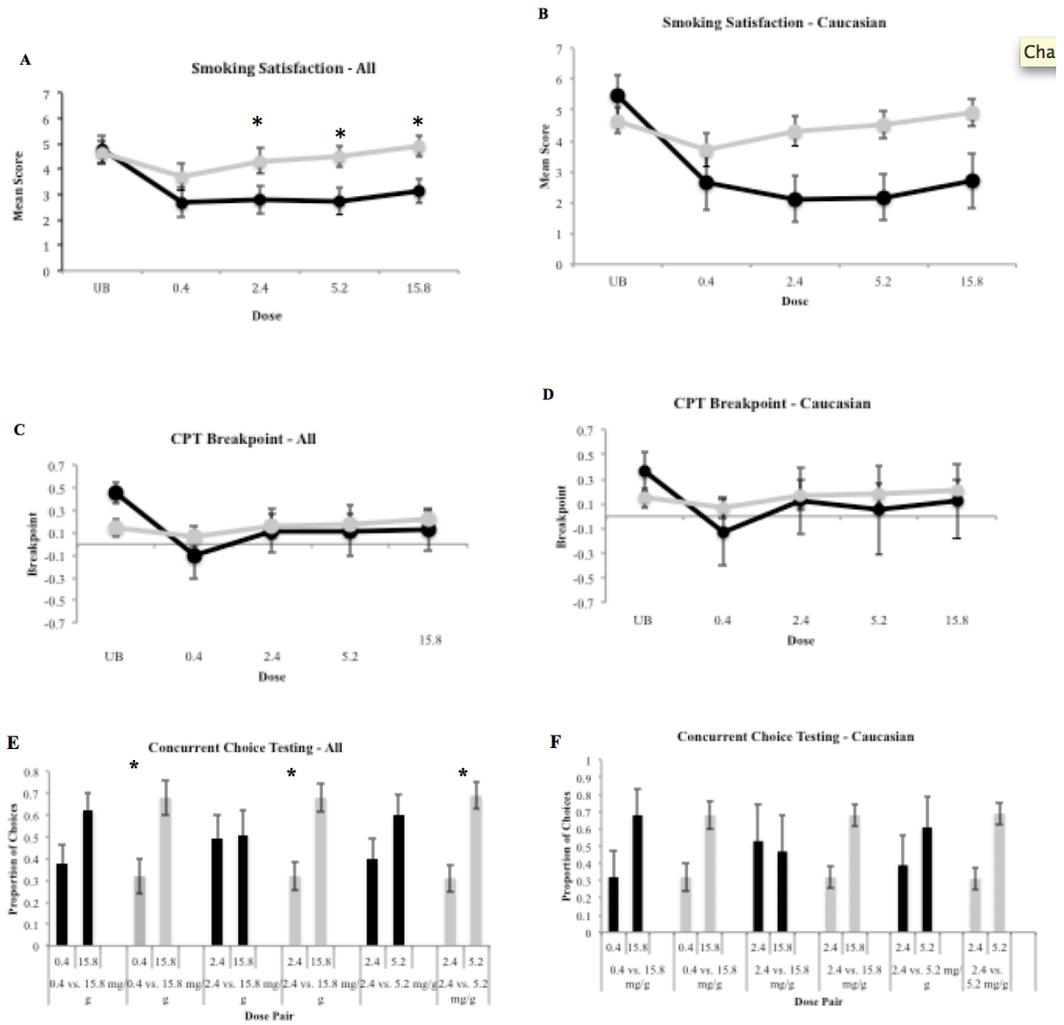


Figure 5. Comparison of Limited Sample for Examination of Effect of Race



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