Validating a Procedure to Assess Declines in Acute Cigarette Self-Administration due to Reductions in Nicotine Content

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The FDA has considered reducing the maximum allowable nicotine content in cigarettes to facilitate quitting in dependent smokers. To potentially inform clinical research on nicotine reduction, this laboratory-based study used a within-subjects forced-choice paradigm to assess dose-related declines in relative nicotine reinforcement in dependent adult smokers (N=37). The aim was to establish the clinically predictive validity of this paradigm by relating findings to results from the Donny et al. (2015) clinical trial on smoking reduction with reduced nicotine cigarettes. In five sessions following overnight abstinence, SPECTRUM research cigarettes varying in nicotine contents (17.4, 11.2, 5.5, 2.3, and 1.3 mg/g; one "NIC" dose per session), were compared to a very low nicotine content cigarette ("VLNC"; 0.4 mg/g). Each session began with four 4-puff exposure trials (2 each NIC or VLNC, identified by letter codes). Assessment of pleasurable sensory perceptions of smoking occurred immediately following each exposure trial. Next were four choice trials in which NIC and VLNC cigarettes were presented concurrently; participants were instructed to take four puffs from any combination of the cigarettes they wanted, totaling 16 choices. Overall, the number of NIC choices and the magnitude of difference in pleasurable sensory perceptions (NIC – VLNC) increased significantly as the nicotine content condition increased. Sensory responses were found to mediate the relationship between nicotine content condition and choice. Differences in choice and sensory responses due to menthol preference and/or ethnicity were also found. However, the pattern NIC choices across nicotine content

conditions were not consistent with the pattern of results observed by Donny et al. (2015), failing to establish clinically predictive validity of the forced choice procedure. Although this within-subjects acute choice procedure did not closely relate to the between-subjects ad lib smoking behavior across weeks in Donny et al., this procedure may combine with other data to suggest a nicotine reduction to ≤2.3 mg/g may attenuate reinforcement.

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1.0 Introduction

Despite a steady decrease in prevalence over the decades following the 1964 United States Surgeon General's Report (U.S. Department of Health and Human Services [USDHHS], 1964), tobacco smoking remains a public health crisis in the United States (US). Smoking prevalence among US adults (≥18 years) has continued to decline in the 21st century—20.9% in 2005 to 14.0% in 2017—but there are still 34.3 million daily cigarette smokers (Wang et al., 2018), and smoking remains the leading cause of preventable mortality in the US, responsible for 480,000 deaths every year (USDHHS, 2014). Action is needed to further reduce the prevalence of tobacco smoking and lessen its burden on public health.

Nicotine is the primary reinforcing constituent in tobacco, largely responsible for initiation and maintenance of smoking behavior (Rupprecht, Smith, Schassburger, Buffalari, Sved, & Donny, 2015; USDHHS, 2014). To mitigate harm from tobacco smoking, the US Food and Drug Administration (FDA) is considering regulation to reduce nicotine content in cigarettes (FDA, 2018). One goal of this proposed regulation is to reduce the nicotine content in cigarettes to a level that would make it easier for established smokers to quit (Benowitz & Henningfield, 1994, 2013, 2018). For context, nicotine contents of the 100 most popular cigarette brands sold in the United States range 13.2 to 29.3 milligrams of nicotine per gram of tobacco filler (mg/g), with an average of 17.2 mg/g (Carmines & Gillman, 2019). Research is needed to identify how much of a nicotine reduction is needed to meet the FDA's goal and provide the public health justification for establishing that new nicotine standard (FDA, 2018). For example, if the maximum nicotine content is set too high, established smokers may continue to struggle with quitting; if set too low, smokers may not find the products acceptable (even if they make quitting easier), which may

promote a black market for cigarettes containing higher levels of nicotine (Berman, Zettler, & Ashley, 2018).

The most compelling data to date—providing support for the potential efficacy of a nicotine reduction policy in reducing tobacco-related harm—comes from a multi-site randomized controlled clinical trial conducted by Donny and colleagues (2015). In their seminal study, Donny et al. assessed smoking reduction in different groups after switching exposure to one level of nicotine content cigarettes (15.8, 5.2, 2.4, 1.3, or 0.4 mg nicotine per gram of tobacco) across six weeks of use in the natural environment. At the end of the study, mean cigarettes smoked per day was similar between the 15.8 and 5.2 mg/g groups, but groups assigned to contents ≤2.4 mg/g smoked significantly fewer cigarettes per day than those in the 15.8 and 5.2 mg/g groups (Donny et al., 2015). In other words, reducing nicotine content in cigarettes to levels ≤2.4 mg/g led to significant declines in self-administration, relative to the 15.8 mg/g cigarette (i.e. that containing a nicotine level typically found in commercial brand cigarettes).

Large-scale randomized controlled clinical trials are often regarded as the ideal source for data used to determine the safety and efficacy of novel interventions (Frieden, 2017; Houle, 2015; Tunis, Stryer, & Clancy, 2003), such as the investigational tobacco products examined in Donny et al. (2015). There are many strengths inherent to this methodology (Frieden, 2017; Houle, 2015). For example, randomizing participants to treatment groups minimizes risk of biased results by equally distributing known and unknown potential confounding variables between groups. Also, use of a control—typically a placebo or usual treatment—allows for better identification of causal relationships. Despite these methodological strengths, randomized controlled trials are resource intensive; these studies typically require large sample sizes, are expensive, and often take many years to plan, implement, and complete (Frieden, 2017).

Shorter-term, lab-based studies may be able to provide similarly valid evidence and inform new clinical trial designs (Frieden, 2017; Tunis, Stryer, & Clancy, 2003). In the context of the present study, a lab-based paradigm assessing relative reinforcement of nicotine across a range of doses may be sensitive enough to identify the reduction in nicotine content needed to detect significant declines in relative reinforcement (used here as an index of cigarette self-administration), somewhat similar to the measure of self-administration examined by Donny et al. (2015). If shown to have such predictive validity, a lab-based within-subjects study, requiring a much smaller sample (and thus shorter and more feasible), could be used to inform design of such trials.

On the other hand, it is not yet clear whether results from lab-based studies of acute relative reinforcement could directly inform design of longer-term clinical trials. There is an underlying assumption that these different research designs would yield comparable results despite divergent methods. To test this assumption, Katz (1990) proposed that the predictive ability of a relative reinforcement paradigm can be validated by relating its results to actual drug use data. Earlier studies have tried to relate results of such lab-based studies of various drugs to indirect clinical evidence from epidemiological survey data, with mixed results—most likely due to a lack of specificity when assessing amount of drug use in the epidemiological data (Katz, 1990). As Katz stated, a better way to establish the predictive validity of an acute lab-based study of relative reinforcement would be to more directly test against results of a clinical trial assessing rates of use for those same drug doses over an extended period of time. This validation approach would rely on assessments of actual drug use rather than broad epidemiological measures used in earlier studies.

The current study aims to establish the predictive validity of a within-subjects lab-based procedure in determining how much of a decline in nicotine content is needed to significantly reduce acute relative reinforcing effects of nicotine. Specifically, I aim to show that differences in acute reinforcement due to changes in cigarettes' nicotine content will be predictive of patterns of self-administration observed in extended clinical study of these cigarettes. To stringently test the clinical implications of this acute lab test, results will be judged valid if consistent with Donny et al. (2015): cigarettes containing <5 mg/g of nicotine will significantly reduce choice (indexing self-administration) relative to that typical of nicotine found in commercially available cigarettes, represented here by one with 17.4 mg/g (Carmines & Gillman, 2019). Described in greater detail below, the within-subjects choice paradigm used in the current study, with its focus on preference between nicotine contents rather than amount of ad lib consumption from each content (as in Donny et al., 2015), may be more sensitive to differences between contents (Perkins, Jacobs, Sanders, & Caggiula, 2002; Shahan, Bickel, Madden, & Badger, 1999).

In the upcoming sections, I will provide a description of recently developed reduced nicotine content (RNC) research cigarettes, followed by a detailed review of Donny and colleagues' clinical trial of these RNC cigarettes. Finally, I will describe commonly used methods to assess acute nicotine reinforcement and how they potentially could be used to assess dose-response effects consistent with smoking behavior measured by Donny et al. (2015), ending with a description of the "forced choice" procedure employed in this validity study.

1.1 Reduced Nicotine Content Cigarettes

Reduced nicotine content (RNC) cigarettes were designed to manipulate nicotine content in the tobacco, to better control how much nicotine is delivered via smoking, compared to previously available "light" or "ultra-light" commercial cigarettes, which manipulated nicotine "yield". Nicotine yield is the amount of nicotine in smoke extracted from a cigarette via smoke machines designed to draw a standardized amount of smoke from the cigarette being tested (Pillsbury, 1996). So-called "light" or "ultra-light" commercial cigarettes were able to alter nicotine yield assessed by the machines through filter ventilation holes or other design aspects (Kozlowski et al., 1994; 2002). However, unlike smoke machines, human smokers can change their smoking behavior (e.g., take deeper puffs, cover ventilation holes, etc.) to alter the resulting nicotine intake despite a fixed nicotine content in the cigarette, reducing the validity of yield as a measure for assessing nicotine delivery (Kozlowski et al., 1994; 2002). With RNC cigarettes, by contrast, smokers cannot easily alter smoking behavior to extract higher levels of nicotine (Benowitz et al., 2012; Kamens et al., 2019), allowing studies using RNC cigarettes to better evaluate dose-response effects due to nicotine via tobacco smoke.

SPECTRUM RNC cigarettes were made available for use in research through the NIDA Nicotine Research Cigarettes Drug Supply Program (https://www.drugabuse.gov/nicotine-research-cigarette-drug-supply-program). These SPECTRUM cigarettes are matched on non-nicotine factors (e.g., branding, paper and filter characteristics, etc.) but differ in the nicotine content of the tobacco they contain (Richter et al., 2016). The nicotine content is determined by mixing varying ratios of conventional nicotine tobacco and tobacco that has been genetically altered and bred to produce low levels of nicotine. The lowest nicotine content SPECTRUM cigarette (0.4 mg/g) contains only the low level nicotine tobacco, the highest content (15.8-17.4

mg/g, varies by batch) contains only the conventional nicotine level tobacco, and the levels between (i.e., 1.3-11.2 mg/g) contain varying blends needed to obtain the target nicotine content (Carmines & Gillman, 2019; Richter et al., 2016).

1.2 Randomized Trial of Reduced-Nicotine Standards for Cigarettes

Donny and colleagues (2015) conducted the first large-scale clinical trial of SPECTRUM cigarettes to assess changes in smoking behavior due to extended use of cigarettes containing moderate to low amounts of nicotine. Results of this trial were intended to provide an empirical foundation to guide potential regulation limiting nicotine content in cigarettes. Eight hundred and forty participants—healthy adult smokers recruited across ten sites nationwide—were randomized to exclusively use one level of SPECTRUM cigarettes for the six-week study period: a control cigarette containing 15.8 mg/g (the conventional nicotine level available in SPECTRUM cigarettes at the time), and cigarettes containing lower levels of nicotine: 5.2, 2.4, 1.3, 0.4 mg/g, or 0.4 mg/g with high tar. All SPECTRUM cigarettes were provided free of charge and in double-blind fashion.

The primary dependent measure was self-reported number of cigarettes smoked in the prior day, reported through a phone-based interactive voice-response (IVR) system where participants used a telephone keypad to respond to verbal prompts. Additional measures of nicotine dependence, biomarkers of nicotine exposure, and 24-hour abstinence-induced withdrawal symptoms were collected in at least one of ten lab visits. At the end of the six-week study, participants assigned to contents ≤2.4 mg/g smoked significantly fewer cigarettes per day compared to the 15.8 mg/g group, thus, demonstrating cigarettes containing ≤2.4 mg/g are less reinforcing than those with contents similar to brands currently available commercially. Additional

measures obtained at week 6 indicated significantly lower levels of total urinary nicotine equivalents, nicotine dependence, and magnitude of withdrawal symptoms when 24-hrs abstinent for groups ≤2.4 vs 15.8 mg/g. Taken together, reducing nicotine content in cigarettes from 15.8 to ≤2.4 mg/g reduced self-administration (as assessed with self-reported cigs/day and biomarkers of nicotine exposure) and nicotine dependence without increasing withdrawal-related discomfort when abstinent.

Results of Donny et al. (2015) provide compelling evidence that reducing the nicotine content in cigarettes to levels at or below 2.4 mg/g will lead to reductions in cigarette consumption in healthy adult smokers. One possible limitation was the use of IVR to assess cigarettes smoked in the previous day. Despite the recency of the smoking behavior being reported, retrospective recall of cigarette consumption has been shown to be affected by digit bias or "heaping", where participants round to multiples of 5's or 10's (Griffith, Shiffman, & Heitjan, 2009; Shiffman, 2009; Wang & Heitjan, 2008; Wang, Shiffman, Griffith, & Heitjan, 2012). Indeed, inspection of the data from Donny et al. (2015) show that groups clustered around 15 or 20 cigarettes per day at week six. Similar studies have used ecological momentary assessment or collected all smoked cigarette butts to corroborate IVR measures of cigarette consumption (Mercincavage et al., 2016, 2018; Shiffman et al., 2018). Direct observation of all smoking would be one way to objectively assess smoking exposure, but this approach might not be feasible in a clinical trial. In contrast, smoking would be able to be directly observed and objectively measured in a short-term lab-based study assessing declines in self-administration due to changes in cigarettes' nicotine content. In the following section, I will discuss commonly used lab-based self-administration research methods which may be able to detect changes in smoking behavior due to reductions in nicotine content.

1.3 Acute Self-Administration Research Methods

Self-administration studies are recognized as the gold standard for assessing drug reinforcement (Bozarth, 1987; Henningfield & Goldberg, 1983; Jones & Comer, 2013; Rupprecht et al., 2015; Sofuoglu & LeSage, 2012). Broadly, self-administration studies measure how much drug an individual consumes (MacKillop & Murphy, 2013). For a drug to be considered reinforcing, it must be self-administered to a greater degree than a vehicle or placebo (Higgins & Hughes, 1998; Sofuoglu & LeSage, 2012). Here, reinforcement is used to describe the relationship between behavior (i.e., drug use) and consequences of that behavior (i.e., increasing likelihood of further drug use; Bozarth, 1987). This behavioral definition of reinforcement does not rely on subjective perceptions of a drug's effects (e.g., 'liking', 'wanting', 'satisfying', etc.) to determine its magnitude of reinforcement. There are a variety of paradigms used in self-administration research (Bozarth, 1987; Carter et al., 2009; Higgins & Hughes, 1998; MacKillop & Murphy, 2013); those most relevant to the current project will be identified and discussed below, followed by review of a study using these methods to assess differences in smoking behavior using SPECTRUM cigarettes in a narrow sample of smokers.

1.3.1 Self-administration paradigms

Commonly used methods for assessing nicotine reinforcement in self-administration research include free choice, operant response, and forced choice procedures (Bozarth, 1987; Carter et al., 2009; Higgins & Hughes, 1998; MacKillop & Murphy, 2013). A number of methods are common across these paradigms, including within-subjects designs, requiring fewer participants to achieve adequate statistical power to detect an effect relative to between-subjects

designs (Carter et al., 2009; Cohen, 1988). Because self-administration of one dose is compared to self-administration of a placebo or another dose, participants typically first sample each available option, either in the same or preceding sessions (Carter et al., 2009). Initial exposures to each dose are necessary to familiarize participants with each available option to inform subsequent self-administration behavior. In clinical studies, doses are often presented in single- or double-blind fashion and are identified by either a letter, number, or color code to maintain blinding to dose (de Wit & Johanson, 1987; Duke, Johnson, Reissig, & Griffiths, 2015; Perkins et al., 1994, 1996, 2017a, 2018; Rukstalis et al., 2005). Also, participants are commonly required to abstain from smoking overnight before experimental sessions (Carter et al., 2009; Perkins et al., 1994, 1996, 2017a, 2018; Rukstalis et al., 2005). In context of the current study, abstinence prior to sessions ensures minimal levels of blood nicotine in all participants and minimizes confounding effects of recent smoking—such as rapid satiation during self-administration testing—which might lead to avoidance of nicotine to prevent toxicity (Carter et al., 2009).

1.3.1.1 Free choice

In a free choice paradigm, participants choose unrestricted, or in ad lib fashion, between concurrently available doses of a drug. The number of doses presented within each session can be two (e.g., placebo vs. active, low vs. high, etc.) or greater (e.g., placebo vs. low vs. high). Participants may choose to abstain or consume as much drug as they would like, over a fixed period of time. Using fixed session durations prevents confounding between drug consumption and duration—if session durations were dependent on continued drug consumption, some participants may choose to abstain or consume minimal amounts of drug to leave sooner (MacKillop & Murphy, 2013).

The main dependent variable in the free choice paradigm is typically the difference in amount (if any) of each drug dose consumed, expressed in absolute number of choices or as a proportion. Variability in the total number of choices in a free choice procedure limits this paradigm from being used to identify differences in "reinforcement" because determining whether one dose was chosen significantly more than another depends on the total number of choices. If that is free to vary between participants, the number of choices for the active drug to be self-administered significantly more than placebo would also vary (even if the proportion of choices were the same). For example, if one participant were to self-administer the active dose fourteen times and choose the placebo six times, their proportion of active choices (70%) would be the same for another participant who chose the active dose seven out of ten times (70%). The binomial probability of active drug choices for the first participant ($p = 0.04$), but not the second participant ($p = 0.12$), would be statistically significant (i.e., $p < .05$), indicating that the difference in number of choices was not due to chance for the first participant only. Using a procedure with a fixed number of total choices would allow for an a priori defined minimum number of choices for a dose to be self-administered reliably more than placebo, consistent across all participants. Lastly, an unrestricted amount of drug is typically provided to the participant for free—there is no financial or behavioral expenditure required to obtain it. Thus, it is possible that consumption may be inflated when using this procedure, relative to others described below.

1.3.1.2 Operant response

Operant self-administration procedures are the most commonly used to assess drug reinforcement, especially in animal models (Rupprecht et al., 2015). These studies require a behavioral response (e.g., lever press, mouse click, button press, etc.) for the participant to gain access to the drug (Higgins & Hughes, 1998). The interval between each presentation of the

reinforcer (i.e., earning a unit of drug) largely depends on the schedule of reinforcement. Studies using a fixed ratio (FR) schedule require a constant number of responses per unit of drug (Murphy & Lupfer, 2014). For example, a study using an FR10 schedule would require a participant click a mouse button ten times, any time they would like over the course of a session, to earn a puff from a cigarette (e.g., a unit of drug). Drug may also be available on a progressive ratio (PR) schedule, which requires a steadily increasing number of responses in order to earn each unit of drug (Murphy & Lupfer, 2014). For example, an initial cigarette puff may require 10 button presses and increase by 50% for each subsequent puff (i.e., "PR50"; 10, 15, 23, 34, 51…). As in the free choice paradigm, different doses can be tested across separate sessions to assess dose-effects on reinforcement. Concurrent schedules, which allow the participant to choose to work for one of two doses simultaneously, may also be used to assess relative reinforcement between doses more directly (Murphy & Lupfer, 2014; Perkins, Jacobs, Sanders, & Caggiula, 2002).

1.3.1.3 Forced choice

In the forced choice paradigm, participants are presented with a pair of doses (or products differing in other ways but identical in appearance) and are instructed to self-administer any combination of the two products according to their preference (Carter et al., 2009; de Wit & Johanson, 1987; Perkins et al., 1994, 1996, 2017a). The total number of choices within each session is typically fixed across all participants. For example, if instructed to take a total of four puffs from some combination of "Cigarette A" or "Cigarette B", a participant could choose one puff from one and three from the other, two from each, or all four from just one of the cigarettes. Generally, the dependent variable is the number or percent of choices for one dose over another. One advantage of the forced choice paradigm is the fixed number of choices. As described above, using a fixed total number of choices allows for a clearly defined minimum difference needed for

one dose to be self-administered to a greater degree than another. Another advantage is that the fixed number of choices is usually kept somewhat low, thus reducing the likelihood that a smoker will become satiated or suffer adverse effects from toxicity, which could lead to differential intake due to avoidance of further intake from one of the options. In the next section, I will discuss application of a choice procedure in recent research of reduced nicotine content cigarettes.

1.4 Research on Acute Relative Reinforcing Effects of Nicotine via SPECTRUM Cigarettes

Higgins et al. (2017) examined preference of SPECTRUM reduced nicotine cigarettes (RNC) in a relatively large sample of smokers considered "vulnerable" to tobacco addiction (N=169; three subgroups characterized by a current affective disorder diagnosis (n=56), history of opioid dependence (n=60), or low SES women (n=53)). All sessions followed overnight abstinence and participants took two puffs from their own brand of commercial cigarette upon arrival. In the first phase of the study, participants were instructed to freely smoke one of four SPECTRUM cigarettes (0.4, 2.4, 5.2, & 15.8 mg/g; one per session, cigarettes identified by letter code). In the next phase, participants were presented with all possible dose pairs (one pair per session) in six 3-hr sessions. Using a hybrid of free choice and operant response methods, participants could choose to abstain or respond on an FR10 schedule to receive two puffs from one of the available cigarettes, up to as many puffs as they would like over the 3-hr session.

Results indicated that when collapsing across all vulnerable population groups, participants chose the higher nicotine content cigarette significantly more than the lower nicotine content, in all dose pairs (Higgins et al., 2017). Choice across dose pairs did not vary due to dependence severity or number of smoking-related chronic health conditions (Higgins et al., 2018; Streck et

al., 2018). However, vulnerability groups differed when choosing between the 2.4 and 0.4 mg/g doses (Higgins et al., 2017). Those with an affective disorder diagnosis chose the 2.4 mg/g dose significantly more than the 0.4 mg/g, whereas there were no differences in choice between these doses for the opioid dependent and low SES women groups. Together, this suggests that certain subpopulations of smokers may have differential sensitivity to nicotine reinforcement, possibly related to having a diagnosed psychiatric disorder.

Using a lab-based procedure involving acute exposure to cigarettes, the Higgins et al. (2017) study addressed an important question of dose-response effects for choice behavior in a narrow sample of smokers with specific characteristics that potentially made them especially vulnerable to tobacco use. As noted by Higgins et al. (2017), results were clearly different from Donny et al. (2015), which found a significant difference in daily self-administration between cigarettes containing 15.8 mg/g versus those containing 2.4 mg/g or below, not between all groups randomized to the different nicotine content cigarettes (as Higgins found). As outlined next, this difference between study results could have been due to the widely differing samples (i.e., healthy adult smokers versus vulnerable populations) or other methodological discrepancies (e.g., acute within-subjects comparisons between all nicotine content levels versus between-subjects comparisons among groups exposed to just one level over an extended period of time).

Methodologically, the statistics used by Higgins et al. did not seem to be well suited for the data. The authors used repeated measures analysis of variance to compare differences in proportion of choices across all dose pairs. This analysis assumes a normally distributed dependent variable (Brase & Brase, 2015); no information was provided on the distribution of choices or the number of participants who chose to abstain in each session (and how abstainers' data were handled). It is likely that count data, which are bounded by zero, would not be normally distributed

(Brase & Brase, 2015). (Higgins was contacted by email on Nov 12, 2018 to provide these data but provided no response.) Thus, count data would be most appropriately analyzed using a statistic specialized for Poisson, binomial, or negative binomial distributions (Zuur, Ieno, Walker, Saveliev, & Smith, 2009).

In summary, results of the lab-based study of smokers considered "vulnerable" to tobacco addiction (Higgins et al. 2017) varied from results reported from a six-week randomized trial with medically and socially typical adult smokers (Donny et al. 2015). In Higgins' lab-based study, reductions in nicotine content decreased relative reinforcement effects of smoking across all dose pairs; each of the higher nicotine content cigarettes was chosen more than any of the lower content cigarettes. In contrast, results from Donny's randomized trial identified significant declines in self-administration between cigarettes containing $\geq$ 5.2 mg/g versus those $\leq$ 2.4 mg/g. These differences were possibly due to methodological factors identified above, including the disparate populations tested.

1.5 Sensory Perceptions of Acute RNC Cigarette Use and Nicotine Reinforcement

Pleasurable "sensory" perceptions of acute drug use (e.g. 'liking', 'satisfying', 'strong', etc.) can be assessed by self-report in self-administration studies to complement behavioral measures of reinforcement but, by definition, do not measure reinforcement per se (Bozarth, 1987; de Wit & Johanson, 1987). Despite this, sensory perceptions provide important interoceptive information to the user which may affect subsequent self-administration behavior. For example, a drug dose with greater pleasurable sensory perceptions may be more likely to be consumed and at greater levels than doses with fewer such effects. While earlier studies have identified dose-

response effects of pleasurable sensory perceptions of acute SPECTRUM RNC cigarette use (Cassidy et al., 2018; Faulkner et al., 2017; Hatsukami et al., 2012; Higgins et al., 2017), there is little research directly associating these sensory perceptions with subsequent smoking behavior.

One study of healthy dependent adult smokers found that the magnitude of difference in positive sensory perceptions of smoking SPECTRUM cigarettes most widely varying in nicotine content (0.4 & 16-17 mg/g) predicted preference for the 16-17 mg/g dose in a forced-choice paradigm (Perkins, Karelitz, & Kunkle, 2018). The likelihood of choosing the 16-17 mg/g cigarette increased with greater increases in positive sensory perceptions of the 16-17 vs. 0.4 mg/g cigarettes. Another recent study of smokers from Higgins et al. (2017) also examined the relationship between choice behavior and positive subjective perceptions of acute use of SPECTRUM cigarettes (Bergeria et al., 2019). As described above, participants were presented with all possible dose options and were free to complete a simple operant task to receive two puffs from one cigarette or another over a three-hour period (Higgins et al., 2017). Researchers found that among vulnerable population subgroups of smokers, greater differences in sensory perceptions were associated with more choices of the higher nicotine content cigarette across all dose pairs. Together, these findings suggest that pleasurable sensory perceptions may be related to the relative reinforcing effect of nicotine, but research is limited to studies of smokers with psychiatric conditions and other vulnerabilities to tobacco addiction (Bergeria et al., 2019), or limited number of nicotine contents compared (Perkins, Karelitz, & Kunkle, 2018). It is possible that forced-choice responding in healthy adult smokers may be related to positive sensory perceptions of smoking RNC cigarettes across a range of nicotine contents, but this has not been directly tested.

1.6 Gaps in the Literature

Nicotine reduction policy may ease tobacco smoking's burden on public health by reducing smoke exposure through decreasing cigarette consumption or by making it easier for established smokers to quit (Apelberg et al., 2018; Benowitz & Henningfield, 1994, 2013, 2018). Randomized clinical trial research has determined that a reduction in nicotine content in cigarettes from current levels (i.e., ~15-17 mg/g) to ≤ 2.4 mg/g is necessary to reduce self-administration in healthy adults already dependent on nicotine (Donny et al., 2015). It is unclear whether shorter-term lab-based studies of relative reinforcement, which may promise a much more efficient and quick method of arriving at similar findings, would be a useful adjunct for such clinical trial research. Specifically, lab-based studies examining dose-response effects of relative reinforcement and subjective perceptions of acute RNC cigarette use will be shown to have relevant clinically predictive validity only by relating those findings to results from clinical trials on smoking reduction with RNC cigarettes. If a shorter-term within-subjects study is able to detect a similar dose-response pattern of preference, or choice, as that found for ad lib self-administration in Donny et al. (2015), this method may be used to inform design of future such clinical trials. It could do so, for examples, by piloting specific components of a study, or as a more cost-effective method (i.e., requiring fewer participants, less cost for research staffing) to gauge likely responses in subpopulations of smokers or to test dose-response effects in novel or investigational tobacco products.

1.7 The Current Study

The main purpose of the current study was to validate the use of a within-subjects forced-choice procedure to determine how much of a decline in nicotine content from levels typically found in commercial brand cigarettes would be needed to significantly reduce cigarette preference (i.e., self-administration). Successful validation of this procedure for this purpose would be expected to result in a dose-response pattern of relative reinforcement consistent with changes in self-administration due to nicotine content reported in a seminal clinical trial by Donny and colleagues (2015). At the end of the six-week trial, Donny et al. found significant differences in the number of cigarettes smoked per day between groups assigned to exclusively use one of six levels of cigarettes varying in nicotine content. Groups assigned to contents ≤2.4 mg/g smoked significantly fewer cigarettes per day than groups assigned to use the 5.2 mg/g cigarette or the "standard" content cigarette of 15.8 mg/g (Donny et al., 2015).

As a first step toward establishing predictive validity of the present forced-choice paradigm, **Aim 1** intended to examine the pattern of choices between a range of higher nicotine content (NIC) cigarettes versus the very lowest content cigarette available—0.4 mg/g (termed here "very low nicotine content" or VLNC). I hypothesized that the number of choices for doses ≤ 2.4 mg/g would be significantly lower than that for contents ≥ 5 mg/g, consistent with the pattern of differences in self-administered cigarettes per day observed by Donny et al. (2015).

A secondary purpose of this study was to relate a composite measure of pleasurable subjective sensory perceptions of acutely smoking RNC cigarettes (as assessed in some earlier RNC studies) to subsequent choice behavior (i.e. relative reinforcement). Clinical substance abuse research typically assumes that a drug is reinforcing because of the acute pleasurable subjective effects it elicits, but little prior research has directly associated these self-report and self-

administration measures (Perkins, Karelitz, & Kunkle, 2018). Thus, **Aim 2** sought to examine whether pleasurable sensory perceptions of acute NIC vs. VLNC cigarette use varies due to nicotine content conditions and whether such responses mediate the relationship between nicotine content and forced-choice behavior, consistent with that choice behavior reflecting positive reinforcement. Results of Aim 2 aimed to extend findings of earlier research on the dose-response effects of acute subjective perceptions of smoking RNC cigarettes to acute relative nicotine reinforcement.

As noted earlier, the fixed number of choices is a strength of the forced choice paradigm. This allows for an a priori defined minimum number of choices needed for one option to be chosen significantly more than the other, based on binomial probability. As another test of differences in choice between doses, **Aim 3** assessed changes in the likelihood of each dose being chosen significantly more than the VLNC (defined as ≥12 of 16 NIC choices) as a function of nicotine content. I hypothesized that the likelihood of doses ≤2.4 mg/g being chosen ≥12 out of 16 times would be significantly lower compared to a standard content cigarette—17.4 mg/g—further suggesting consistency of results from this acute choice test with significant changes in daily smoking between groups differing in cigarette nicotine contents groups reported by Donny et al. (2015).

2.0 Method

2.1 Overview

The current study used a within-subjects design to assess dose response effects on acute subjective perceptions of smoking RNC cigarettes and self-administration behavior in a sample of adult dependent smokers. Eligible participants completed six, 3-hour sessions: an introductory session and five experimental sessions (all following 12 hours of abstinence). All experimental sessions were identical, varying only in the nicotine content of the SPECTRUM cigarette (1.3, 2.3, 5.5, 11.2, and 17.4 mg/g; "NIC" dose) being compared with the 0.4 mg/g. Cigarettes were identified by letter code specific to the session, with order of the higher nicotine content cigarettes counter-balanced across sessions. During the introductory session, participants provided informed consent, completed screening questionnaires, and were introduced to the study procedures (but using their own brand of cigarette vs. 0.4 mg/g very low nicotine content or "VLNC" dose). All smoking was done through a smoking topography device, with timing and duration of the puffs controlled (i.e., guided) by instructions presented via computer (Perkins & Karelitz, 2019).

Each session began with participants providing an expired-air carbon monoxide sample to confirm abstinence, followed by completion of self-report measures of nicotine withdrawal symptoms and craving. Next, were four "exposure" (or "sampling") trials during which participants took four puffs from each cigarette in a fixed order (VLNC, NIC, NIC, VLNC), one cigarette every 20 mins. Sensory perceptions of smoking were collected after each exposure trial so that responses were to 4 puffs from only one cigarette or the other. Unrelated to the choice procedure, participants also completed a simple computer task—Apple Picker—assessing

reinforcement enhancing effects of nicotine following exposure trials 2, 3, and 4. (This computer task was added to take advantage of the downtime between exposure trials to initially pilot nicotine dose response effects on enhancing reinforcement of non-drug related reinforcers. The Apple Picker task and its use in assessing reinforcement enhancing effects of nicotine have been described in detail elsewhere [Perkins, Karelitz, Boldry, 2017]; no additional procedures as part of this pilot testing occurred once the forced-choice trials began.) Last were four forced-choice trials—one every 10 mins—to assess acute relative reinforcement, during which participants were presented with both cigarettes and instructed to take four guided puffs from any combination of the cigarettes they would like. All procedures were reviewed and approved by the University of Pittsburgh Institutional Review Board (IRB #PRO13060083).

2.2 Inclusion and Exclusion Criteria

Eligible participants were those who have smoked ≥5 cigarettes per day for the past 12 months, were not interested in quitting, and met DSM-V criteria for tobacco dependence (American Psychiatric Association, 2013). Exclusion criteria included current psychiatric diagnosis, intending to quit soon, current use of psychiatric or smoking cessation medications, current nicotine replacement therapy use, pregnancy, and currently nursing mothers. These criteria were chosen to obtain a sample that generally reflected the non-treatment seeking smokers in Donny et al. (2015). All participants were recruited from the Pittsburgh area using flyers and ads on Craigslist and Facebook

2.3 Procedure

2.3.1 Telephone eligibility screen

Potential participants initially responded to study advertisements via telephone or Qualtrics online pre-screening. All were then contacted via telephone by study staff and presented with a brief overview of the study followed by a 10-minute phone interview to determine eligibility. Those eligible were provided additional details about the study and scheduled for their introductory session.

2.3.2 Introductory session

Participants were instructed to abstain from all nicotine and tobacco products for 12 hours prior to this session and to bring an unopened pack of their preferred brand of cigarettes. An unopened pack was necessary to ensure there would be enough cigarettes to be used in the introductory session. Informed consent was obtained upon arrival, followed by confirmation of 12-hr abstinence from combusted tobacco products via expired-air carbon monoxide (CO) sample ≤10 ppm (Benowitz et al., 2019; SRNT Subcommittee on Biochemical Verification, 2002). Female participants provided a urine sample to test for pregnancy; none of the pregnancy tests were positive. All participants completed questionnaires of smoking and demographic information before learning and practicing study procedures (described in detail below). Cigarettes used in this session came from the unopened pack provided by the participant and the 0.4 mg/g "VLNC" dose provided by the researcher.

2.3.3 Experimental sessions

A visual overview of the session procedures is presented in Figure 1. As in the introductory session, participants were instructed to abstain from nicotine and tobacco products for 12 hours before all experimental sessions to ensure minimal blood nicotine levels prior to testing in all participants, thus minimizing risk of nicotine satiation during the choice procedure trials. An expired-air CO sample was collected upon arrival, those with CO >10 ppm or reporting any smoking during the abstinence period were rescheduled. Next, participants completed baseline assessments of craving and withdrawal, as a manipulation check (see Preliminary Analyses subsection in Analysis Plan below).

Once the baseline forms were completed, participants were told the letter codes corresponding to the 0.4 mg/g versus higher nicotine cigarettes specific to the day ("A" and "B" for session 1, "C" and D" for session 2, "E" and "F" for session 3, etc.). Specifically, participants were told "You will be given two different cigarettes, at least one of which will contain nicotine. They will be called cigarette 'A' and cigarette 'B'". Next, was the first of four exposure trials, one every 20 minutes. The exposure trials were necessary to allow participants to sample the cigarettes to inform choice later in the session. During each exposure trial, participants took four puffs from one of two SPECTRUM cigarettes through a smoking topography device, guided by instructions presented on a computer screen (described in greater detail below). Immediately after taking the puffs, participants completed a sensory rating form (see Appendix). Cigarettes were presented in a fixed order in these four exposure trials across all sessions (VLNC, NIC, NIC, VLNC) to provide adequate exposure to inform subsequent choice, while also minimizing carryover effects for initial sampling of each cigarette. Earlier research has shown that differences between cigarettes varying

in nicotine content are perceived to be greater when compared sequentially within a single session versus independently in separate testing sessions (Perkins et al., 2002).

As briefly noted above, and unrelated to the choice procedure, participants engaged in an operant computer task during the downtime between exposure trials. The task, Apple Picker (described in more detail elsewhere; Perkins, Karelitz, & Boldry, 2017), involved simple responding on a keypad to obtain brief 30-sec clips of audio (music), video, or pictorial stimuli only for as long as desired, for up to a maximum of 15 mins. This pilot was aimed at assessing the reinforcement enhancement effects of nicotine via SPECTRUM cigarettes. This computer task occurred following exposure trials 2, 3, and 4; all choice procedures specific to the current study followed the final computer task.

These exposure trials were followed by four forced choice trials, one every 10 minutes. In the forced choice trials, both cigarettes were presented concurrently. The researcher inserted each cigarette into separate smoking topography devices labeled with their respective letter code from the exposure trials. Participants were instructed to first light both cigarettes (without taking a puff) and then take four puffs from any combination of the cigarettes based on their preference (e.g., 2 from each, 1 from one and 3 from other, or all 4 from one cigarette). As in the earlier trials, timing and duration of each puff was guided by instructions displayed on a computer screen and confirmed by CReSS-obtained smoking topography data.

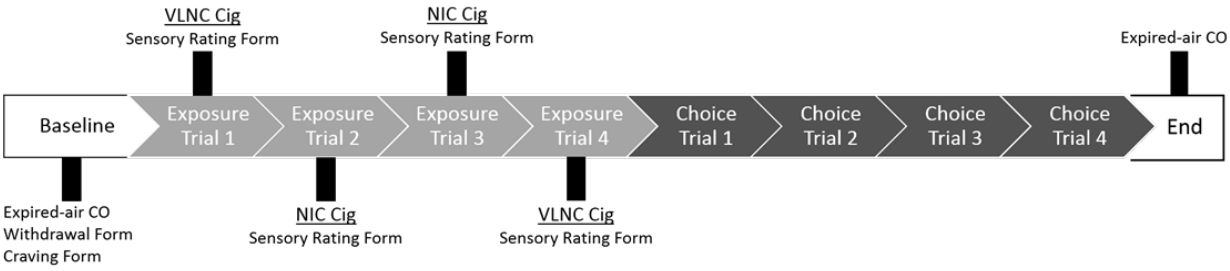

Figure 1 Format of experimental sessions

2.3.4 Research cigarettes

SPECTRUM brand cigarettes (22nd Century Group http://www.xxiicentury.com) differing in nicotine content (see Table 1) were used. Menthol and non-menthol cigarettes were provided based on participant's self-reported preference. Given the very slight difference in nicotine content between menthol and non-menthol cigarettes, the mean content between menthol and non-menthol cigarettes were used to describe each level. These specific nicotine contents were chosen to mostly to match those used in Donny et al. (2015). The 11.2 mg/g cigarette—which was not tested in Donny et al.—was included to anticipate a potential outcome where choice for all contents ≤5.5 mg/g were significantly lower than the number of choices for the 17.4 mg/g. In this case, having an additional level between 5.5 and 17.4 mg/g would narrow the nicotine content gap and may identify a threshold where choice was significantly greater than lower contents but similar to that of a standard content cigarette—17.4 mg/g.

Table 1 Nicotine, menthol, and tar information for SPECTRUM cigarettes by nicotine and menthol status

	Menthol			Non-menthol		
Dose Label	Nicotine Content (mg/g)	Menthol Content (mg/g)	Tar Yield (mg/cig)	Nicotine Content (mg/g)	Menthol Content (mg/g)	Tar Yield (mg/cig)
VLNC	0.39	1.08	8.1	0.44	—	8.4
1.3 mg/g	1.24	2.08	8.5	1.29	—	8.1
2.3 mg/g	2.26	1.89	8.9	2.27	—	9.1
5.5 mg/g	5.65	1.46	8.3	5.33	—	8.6
11.2 mg/g	11.67	0.91	9.8	10.81	—	13.5
17.4 mg/g	16.14	1.51	10.6	18.66	—	10.1

Note. Dose labels are the mean nicotine content between menthol and non-menthol cigarettes; mg/g is milligram per gram of tobacco; mg/cig is milligram per cigarette; VLNC is very low nicotine content. All values provided by RTI International.

The lowest available dose, 0.4 mg/g, was presented concurrently with each of the higher doses during forced-choice trials across sessions. Although a true "placebo" tobacco cigarette would be ideal, SPECTRUM is not available in a 0 mg/g nicotine version, likely because mandating nicotine-free cigarettes is prohibited by the Family Smoking Prevention and Tobacco Control Act (FSPTCA). Comparing against the lowest available dose was necessary to conduct a choice procedure, so that only the nicotine content varied between the two cigarettes being compared.

Comparisons among each permutation of nicotine content pairs (as in Higgins et al., 2017) was not used in the current study for a few reasons. First, it would have increased the number of sessions from six to sixteen, increasing the burden of participation and making it harder to recruit and retain participants. It is also possible that concurrent within-session smoking of the higher nicotine levels participants could cause some participants to become satiated, leading to actively avoiding puffs from the higher nicotine content cigarette in later choice trials. Lastly, comparing each content against the same 0.4 mg/g alternative allows for a much clearer interpretation of results, as the point of comparison is fixed across each of the nicotine conditions.

2.3.5 Control of smoke exposure

All puffing was guided by instructions presented to the participant on a computer monitor. As shown in Figure 2, participants were instructed to "Get Ready" (2 sec) and then "Put the Mouthpiece to your lips" (2 sec), then "Inhale" (2 sec), "Breathe in and hold" (2 sec), and "Exhale" (2 sec), followed by "In a moment you'll do that again. Wait for instructions" (20 sec). The instructions ended following the last puff of each trial. Smoking using this procedure has been shown to result in consistent exposure (i.e., puff volume) within and between cigarettes varying in nicotine content (Perkins & Karelitz, 2019).

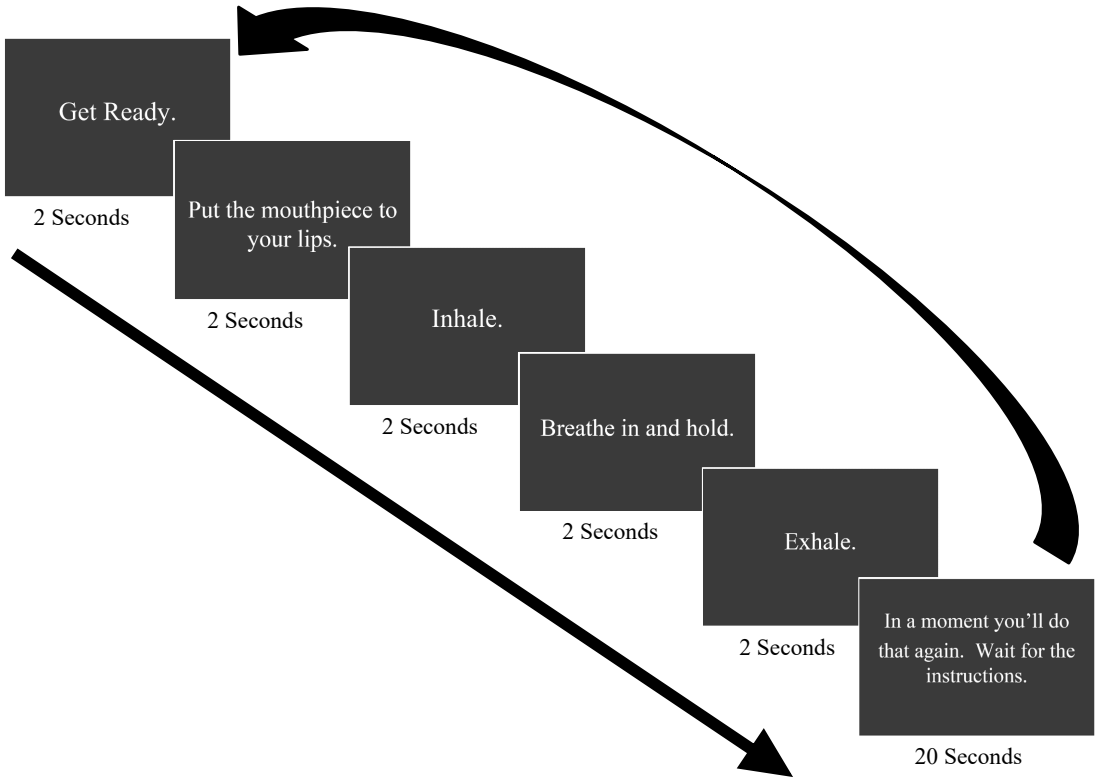

Figure 2 Instructions presented to participants on the timing of each step in taking one puff, displayed on a computer monitor. (From Perkins & Karelitz, 2019)

2.4 Primary Measures

2.4.1 Positive sensory perceptions of smoking

Acute positive sensory perceptions of each acute cigarette smoking exposure were measured immediately after the last puff taken in the four exposure trials, with self-report items on a 0-100 visual analog scale (Appendix). Items of interest asked how much "liking", "satisfaction", "nicotine", and "flavor" was experienced, and how "strong" the cigarette was. These measures have been found to be sensitive to differences in nicotine content (Faulkner et al., 2017; Perkins et al., 2002, 2006, 2018). A composite positive sensory response—the Acute Cigarette Perception (ACP) measure—was calculated as the mean of the "liking", "satisfaction", "nicotine", "flavor", and "strong" items. Earlier research using this composite measure found that it was positively associated with choice for 17 mg/g, when presented concurrently with a 0.4 mg/g alternative (Perkins et al., 2018). Difference scores (NIC – VLNC) were calculated for each sensory response item by nicotine content to separate effects of nicotine from smoking behavior. The difference in sensory perceptions between NIC and VLNC were used in analyses described in detail below. (Additional items collected on this form, including measures of "smooth", "harsh" and "similar to your own brand", are not part of the ACP and were not included in analyses of positive sensory perceptions of smoking which were focused on sensory perceptions items relating to positive reinforcement.)

2.4.2 Self-administration during forced choice procedure

The number of puffs taken from each cigarette (VLNC & NIC) during the subsequent four forced choice trials was measured using smoking topography devices. Study staff inserted each cigarette into its own portable Clinical Research Support System (CReSS; Borgwaldt KC, Inc., Richmond VA), which recorded the number of puffs taken from each cigarette (as well as other smoking topography measures described in greater detail below). The absolute number of puffs for the higher nicotine content cigarette (NIC) used in analyses.

2.5 Secondary Measures

2.5.1 Craving

Craving for cigarettes was assessed upon arrival using the 4-item Questionnaire of Smoking Urges (QSU-4; Carter & Tiffany, 2001). Participants responded using a 0-100 visual analog scale, consistent with other studies using this measure (Conklin et al., 2015, 2018, Perkins, Karelitz, & Michael, 2017, Sayette et al., 2000). A composite craving score was computed by taking the mean of all four items. The QSU-4 has been shown to have high levels of internal consistency, with Cronbach's alpha values ranging .82 to .94 (Litvin, Kovacs, Hayes, & Brandon, 2012).

2.5.2 Withdrawal

Severity of nicotine withdrawal symptoms were measured upon arrival using the Minnesota Nicotine Withdrawal Scale (MNWS; Hughes & Hatsukami, 1986). The MNWS is an eight-item self-report scale, measured on a 0-100 visual analog scale. A single withdrawal score was calculated as the mean of all eight items. This measure has been found to have high internal consistency (Cronbach's alpha $\geq$0.85), stability in measurement over time (test-retest correlation of 0.71), and high construct validity (Etter & Hughes, 2006).

2.5.3 Smoking topography

All cigarettes were smoked through a portable CReSS smoking topography device. This device collects several measures of smoking topography for each puff (e.g., volume, duration, interpuff interval, etc.), which was converted to composite or mean values consistent with prior research to allow comparisons between cigarettes (Conklin et al., 2015, 2018; Perkins et al., 2011, 2012, 2019; Shiffman, 2018a, 2018b; Strasser et al., 2004). Topography measures included total puff volume (ml), total number of puffs, and mean puff volume (ml). These variables were collected to confirm consistency in smoking exposure across SPECTRUM cigarettes varying in nicotine content and to record the number of VLNC and NIC puffs during forced-choice procedures.

2.6 Analysis Plan

2.6.1 Preliminary analyses

All analyses were performed using SPSS 25.0 (IBM, Chicago, IL), with alpha set to 0.05. Baseline craving and withdrawal scores were compared across nicotine conditions using separate linear mixed models (LMM), with a random intercept and fixed effects for the intercept and nicotine condition. These analyses confirmed consistent within-subjects levels of overnight abstinence induced craving and withdrawal symptoms upon arrival to each session. Difference in exposure trial puff volumes (NIC – VLNC) across nicotine content conditions were compared using LMMs to confirm consistent smoke exposure.

2.6.2 Aim 1 analyses: Choice data

Generalized linear mixed models (SPSS GENLINMIXED command) using a Poisson probability distribution and log link were used in all analyses of choice data. Assuming a Poisson distribution makes this analysis appropriate for use with count data, which consists of non-negative, discrete integers (Garson, 2020). Generalized linear mixed models (GLMM) are preferred over general linear models (e.g., repeated measures analysis of variance) when the dependent variable is not normally distributed (Brase & Brase, 2015; Garson, 2020). As noted earlier, count data is often not normally distributed and—when not zero-inflated—should be analyzed using a statistic specialized for a Poisson distribution (current data were not zero-inflated—only 3 out of 181 choices [1.7%] were zeroes; Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Further, GLMMs, unlike general linear models, do not require consistent number of

measurements across groups and is able to account for variance occurring at lower- and higher-levels of observation. GLMM analysis is typically used to examine data arranged in a hierarchical structure (Ciarleglio & Makuch, 2007; Raudenbush & Bryk, 2002; Singer & Willett, 2003). Hierarchical data consists of lower-level observations nested within higher-level groups (the higher-level groups can also be nested within even higher-level groups and so on). For the current study, lower-level within-session forced-choice trials were nested within higher-level nicotine content conditions.

Initial GLMM analysis examined influences on the difference in choice at the lower-level (i.e., between trials), upper-level (i.e., between nicotine contents), and cross-level interaction where patterns of choosing across forced-choice trials may vary as a function of nicotine content (Mathieu, Aguinis, Culpepper, & Chen, 2012). This analysis determined whether the number of NIC puff choices varied systematically across within-session trials and as a function of the nicotine content condition. Specifically, this analysis was run to rule out the possibility that participants avoided the higher nicotine content cigarette due to satiation over the course of the four choice trials, which may have occurred at higher (but not lower) nicotine content conditions. This model included a random intercept and fixed effects for the intercept, nicotine condition, and trial. Discussed further in the Results section, there were no significant effects for the main effect of trial or for the trial by nicotine condition interaction, so choice data were collapsed and summed across trials for all further analyses.

For the primary analysis, GLMMs were used to assess change in the number of NIC puff choices across nicotine content conditions. This model included a random intercept and fixed effects for the intercept and nicotine condition. Several post-hoc exploratory models were also estimated. Exploratory factors included number of cigarettes smoked per day, sex, menthol

preference, and ethnicity (Caucasian vs. African American/Black). These factors were specifically chosen as they have been previously identified as research priorities for studies assessing behavioral and sensory responses to using reduced nicotine content cigarettes (Hatsukami, Benowitz, Donny, Henningfield, & Zeller, 2013). Cigarettes per day was included as a continuous time-invariant covariate; additional models separately assessed moderating effects of sex, menthol preference, ethnicity (African American/Black or Caucasian), and their respective interaction with nicotine content condition. Significant interactions with nicotine condition were decomposed with post-hoc tests, where models were run separately for each level of the categorical moderator.

2.6.3 Aim 2 analyses: Sensory data

Analyses relating to Aim 2 focused on the relationship between positive sensory perceptions of acute RNC cigarette use and subsequent choice behavior. First, separate LMM analyses were used to assess dose-response effects for the self-reported acute positive sensory perceptions: composite ACP, 'Liking', 'Satisfying', 'How Much Nicotine', 'Strong', and 'Flavor'. Difference scores (NIC – VLNC) of cigarette ratings obtained during the exposure trials were compared across the five levels of nicotine content. Menthol preference was also explored in post-hoc analyses as a potential moderator of nicotine content effects on sensory perceptions by inclusion of menthol as a between-subjects factor and the interaction of menthol by nicotine content condition.

The next set of analyses were intended to extend earlier findings by using linear mixed effects mediation analysis to determine whether the magnitude of difference in the ACP composite measure mediated the relationship between forced choice responding and nicotine content conditions. Mediation occurs when the independent variable's (X) effect on the dependent variable

(Y) occurs through the independent variable's effect on the mediator variable (M) (Hayes, 2015; Hayes & Rockwood, 2019). The mediator can be considered the mechanism through which the independent variable is able to affect the dependent variable (Hayes, 2015; Hayes & Rockwood, 2019). This process is visualized using examples from the current study in Figure 3 below; path 'a' is the effect of nicotine content condition on the ACP, path 'b' is the effect of ACP on the number of NIC choices, and path 'c′' is the direct effect of nicotine content condition on the number of NIC choices. The product 'ab' is termed the "indirect effect", which is the effect of nicotine content condition on choice through the ACP (i.e., the product of coefficients method; MacKinnon et al., 2002). The linear mixed effects mediation analysis was performed using the MLmed SPSS macro (Hayes, 2015; Hayes & Rockwood, 2019; Rockwood, 2017; downloaded from https://njrockwood.com/mlmed), using a random intercept, random slopes for the nicotine condition variable, and a diagonal covariance matrix. All mediation effects were estimated using a restricted maximum likelihood (REML) estimator and 10,000 Monte Carlo samples to obtain 95% confidence intervals for the estimates.

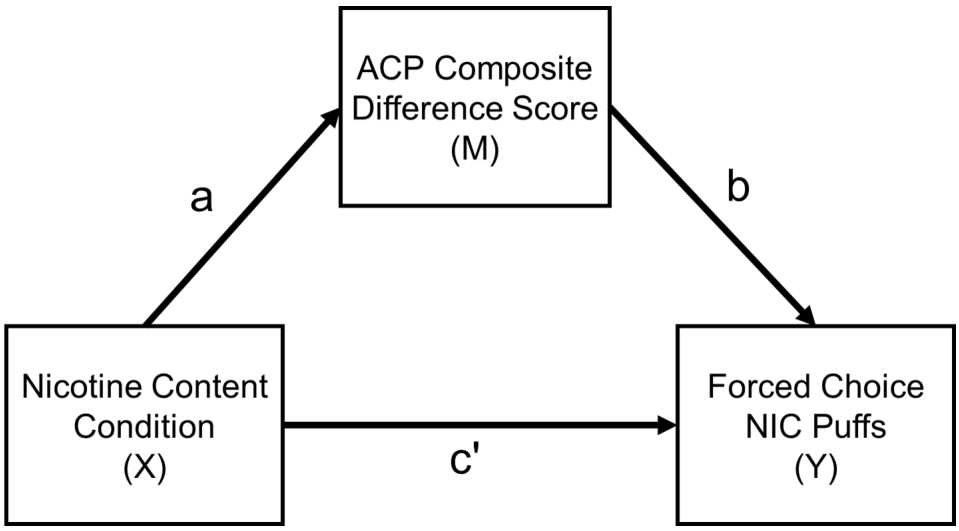

Figure 3 Conceptual model of the mediation analysis. Path 'a' is the effect of nicotine content condition on ACP; Path 'b' is the effect of ACP on forced choice; Path 'c´' is the direct effect of nicotine content condition on forced choice.

An additional moderated mediation model was run using MLmed, assessing the moderating effect of menthol preference on path 'a' (now path 'a_1'; visualized in Figure 4). This model built upon the previous by testing whether the mediation process described above varied as a function of menthol preference (W). Here, the moderator acts to set the context under which mediation occurs through path 'a_2'. The index of mediated moderation is the product of the effect of the interaction between the independent (X) and moderator (W) variables (X*W, path a_3 not shown in Figure 4) on the mediator (M) and path b (the effect of the mediator on the dependent variable): a_3*b (Hayes, 2015; Hayes & Rockwood, 2019). To put this in clearer terms, an example of moderated mediation in context of the current study would be a mediation effect of ACP on the relationship between nicotine content condition and choice being present for non-menthol but not menthol smokers.

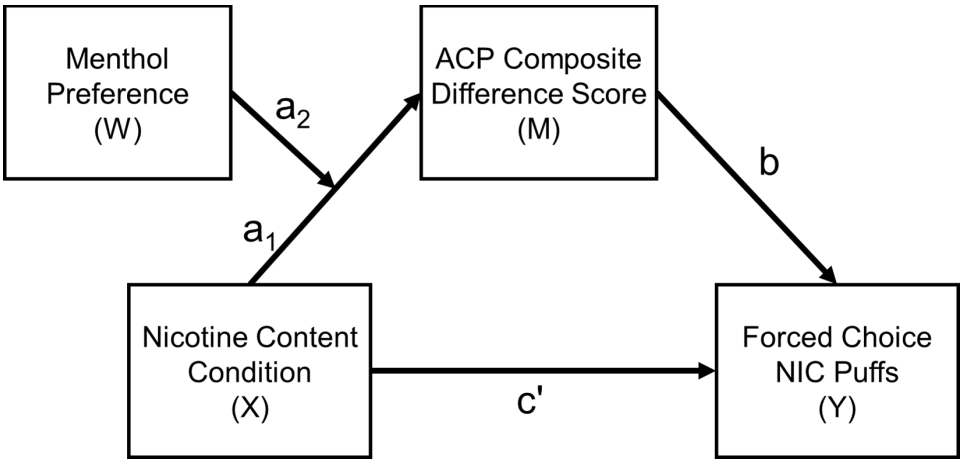

Figure 4 Conceptual model of the moderated mediation analysis.Path 'a$_1$' is the effect of nicotine content condition on ACP; Path 'a$_2$' is the moderating effect of menthol on the relationship between nicotine content condition and ACP; Path 'b' is the effect of ACP on forced choice; Path 'c′' is the direct effect of nicotine content condition on forced choice.

2.6.4 Aim 3 analyses

Aim 3 analyses assessed dose-response effects on the likelihood of individual participants self-administering the NIC dose significantly greater than the VLNC. For a NIC dose to be self-administered significantly more than the VLNC dose, it must have been chosen ≥12 out of the 16 total forced choice puffs. This threshold was determined a priori using the formula for binomial probability (Brase & Brase, 2015):

$$b(x; n, P) = \left\{\frac{n!}{[x!\,(n-x)!]}\right\} * \; P^x * (1-P)^{n-x}$$

In this formula, *n* is the total number of choice opportunities, *x* is the number of NIC dose choices, *P* is the probability of choosing the NIC dose for an individual choice opportunity, and *b* is the binomial probability (Brase & Brase, 2015). In the present study, there were 16 total choice

opportunities (n) with a 0.50 probability of choosing either dose (NIC or VLNC; P), resulting in the formula:

$$b(x; 16, 0.50) = \left\{\frac{16!}{[x!\,(16-x)!]}\right\} * \ 0.50^{x} * (1-0.50)^{16-x}$$

Thus, ≥12 NIC choices (x) would result in binomial probability (b) less than 0.05:

$$b(12; 16, 0.50) = \left\{\frac{16!}{[12!\,(16-12)!]}\right\} * \ 0.50^{12} * (1-0.50)^{16-12}$$

$$b(12; 16, 0.50) = 0.028$$

The absolute number of choices for the NIC cigarette was converted into a binary variable, with '1' indicating ≥12 NIC choices and '0' for <12 NIC choices. A GLMM analysis tested for a dose-response effect for the likelihood of meeting the ≥12 criterion. The GLMM analysis assumed a binary logistic distribution for the dichotomous criterion variable and used a logit link. The 17.4 mg/g content served as the reference group to further confirm consistency in results with Donny et al. (2015).

3.0 Results

3.1 Participants

A total of sixty people attended the introductory session and provided informed consent; fourteen did not meet inclusion criteria (1 smoked <5 cigs/day, 2 were not DSM-V dependent, 11 had CO > 10 ppm) and were excluded. Nine other participants who did meet inclusion criteria did not show up for any additional sessions beyond the introductory session, resulting in a final sample of 37 (20 male, 17 female). Of the 37 participants, two had incomplete data due to moving out of the area before being able to complete all sessions—one participant was missing data for one session (5.5 mg/g condition) and the other is missing data for three sessions (5.5, 11.2, and 17.4 mg/g conditions). All available data were used in the analyses, resulting in final sample sizes per nicotine condition: 37 for 1.3 mg/g, 37 for 2.3 mg/g, 35 for 5.5 mg/g, 36 for 11.2 mg/g, and 36 for the 17.4 mg/g condition.

Sample demographics by menthol preference are presented in Table 2. Overall, the mean (SD) age was 37.1 (11.5) years; they smoked an average of 13.2 (4.8) cigarettes per day, smoking at their current rate for 11.0 (10.1) years, with a mean nicotine content of their reported usual brand of 17.9 (1.9) mg/g (Carmines & Gillman, 2019). There were almost equal numbers of smokers who preferred non-menthol (n=17, 45.9%) and menthol (n=20, 54.1%) cigarettes. The sample as a whole was moderately nicotine dependent, with a mean Fagerström Test of Nicotine Dependence (FTND) score of 4.4 (2.0). Sample characteristics did not vary between sexes, but menthol preferring smokers were more likely to be African American or Black (n = 14) than Caucasian

(n=4), $\chi^2(1) = 16.89$, p = 0.00004 (two additional menthol smokers were Hispanic and more than one ethnicity).

Table 2 Participant demographics by menthol preference

	Menthol (n=20)	Non-menthol (n=17)	Overall (n=37)
Age	37.9 (±11.0)	36.1 (±12.4)	37.1 (±11.5)
Sex			
Male	9	11	20
Female	11	6	17
Ethnicity			
Caucasian	4	14	18
African American/Black	14	1	15
Hispanic	1	0	1
More than one	1	2	3
Cigarettes per day	12.0 (±4.7)	14.7 (±4.6)	13.2 (±4.8)
Own brand nicotine content (mg/g)	18.1 (±1.3)	17.7 (±2.5)	17.9 (±1.9)
Own brand menthol content (mg/cig)	5.1 (±0.4)	—	—
FTND	4.8 (±2.0)	3.9 (±2.1)	4.4 (±2.0)

Note. Values are means (±standard deviation) or frequencies; mg/g is milligram of nicotine per gram of tobacco; mg/cig is milligram of menthol per cigarette.

3.2 Preliminary Analyses

Means and standard errors for baseline assessed expired-air CO, MNWS withdrawal, and QSU-4 craving are presented in Table 3. As expected, there were no significant differences across sessions for any of these measures (F's ≤ 1.40, *p*'s ≥ 0.26). Similarly, there was no main effect of nicotine content condition on the difference (NIC – VLNC) in exposure trial puff volume, F(4,172)

= 0.45, p = 0.77. Together, these results show consistent overnight abstinence induced effects and sampling trial smoke exposure across all nicotine content conditions.

Table 3 Means, standard errors, and F-values for baseline measures

	Nicotine Content Condition											
	1.3 mg/g		2.3 mg/g		5.5 mg/g		11.2 mg/g		17.4 mg/g			
Variable	M	SE	M	SE	M	SE	M	SE	M	SE	F	p
Expired-air CO (ppm)	5.60	0.41	5.57	0.41	5.26	0.41	5.84	0.41	5.29	0.41	0.86	0.49
MNWS withdrawal	27.72	3.23	29.09	3.23	31.89	3.26	28.82	3.25	26.56	3.25	1.33	0.26
QSU-4 craving	74.08	3.92	74.18	3.92	74.26	3.97	74.78	3.95	73.71	3.95	0.03	0.99

Note: F is for effect of nicotine content condition; degrees of freedom of (4, 176) for all F's; MNWS and QSU measured on 0-100 VAS.

3.3 Aim 1 Analyses

3.3.1 Initial test of trial-level effects on choice

There was a significant main effect of nicotine condition, F(4, 704) = 2.62, p = 0.03, indicating that the number of NIC puff choices differed by nicotine condition when averaged across trials. The main effect of trial and the interaction of trial by nicotine condition were not significant—F(3, 704) = 0.64, p = 0.59 and F(12, 704) = 0.22, p = 0.99, respectively. In sum, choice varied due to nicotine condition but not across trials. These results indicate stability of responding within condition, supporting the notion that participants were not avoiding the higher nicotine content cigarette due to satiation as the choice trials progressed.

3.3.2 Main effect of nicotine condition on choice

As mentioned above, all further analyses of choice responding used the session-level summed number of NIC choices (out of 16 total) for each condition. There was a significant main effect of nicotine content condition on the number of NIC puff choices, $F(4, 176) = 2.62$, $p = 0.04$. As illustrated in Figure 5, NIC choices increased with greater nicotine content. Also shown in Figure 5, the number of NIC choices was significantly greater than eight (i.e., none of the 95% CI's overlapped with eight) across all nicotine conditions. As there were 16 total puffs per session, choosing the NIC cigarette eight or fewer times would have meant an equal or greater number of puffs for the VLNC cigarette (0.4 mg/g). Thus, each of the higher nicotine content cigarettes was chosen significantly more than the VLNC cigarette (i.e., significantly greater than eight). Pairwise comparisons indicated that the number of NIC choices for the 17.4 mg/g group was significantly greater than that for both the 2.3 and 1.3 mg/g groups, as hypothesized. The number of NIC choices for 2.3 mg/g was marginally lower than that of the 5.5 condition and significantly lower than the 11.2 mg/g condition.

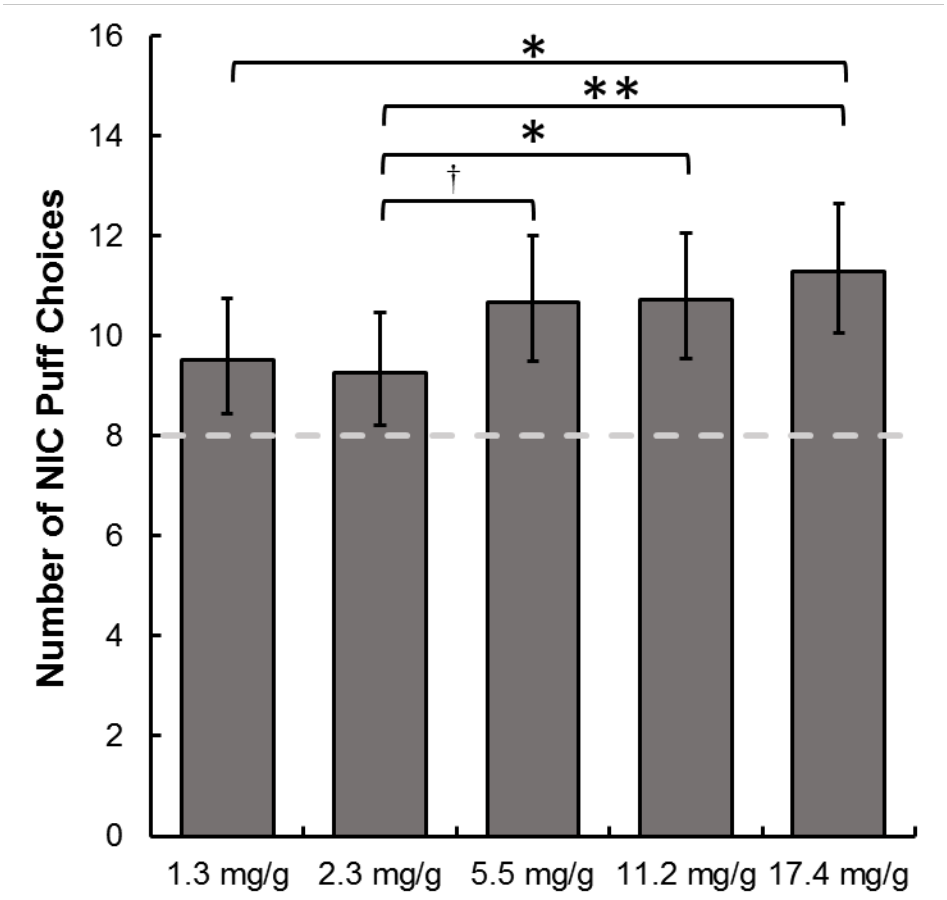

Figure 5 Mean number of NIC puff choices by nicotine content condition. Vertical bars represent 95% confidence intervals. Horizontal bars indicate significant pairwise differences between nicotine content conditions. Horizontal dashed line at 8 indicates no difference in choice from VLNC. **p<.01, *p<.05, †p<.10.

3.3.3 Exploratory post-hoc analyses

Separate exploratory post-hoc analyses were run to assess whether choice behavior across nicotine content conditions varied due to the number of cigarettes smoked per day, sex, menthol preference, and ethnicity (African American/Black and Caucasian only). Each factor (and the interaction with nicotine content condition for the dichotomous measures) was added to the generalized linear mixed model as fixed-effects.

When controlling for cigarettes smoked per day, the main effect of nicotine content condition was still significant, $F(4, 175) = 2.61$, $p = 0.04$, but the main effect of cigarettes per day was not, $F(1, 175) = 0.16$, $p = 0.69$. Therefore, the effect of nicotine content condition on the number of NIC choices was consistent across all levels of cigarettes smoked per day.

In the model adding factors of sex and the sex by nicotine condition interaction, the main effect of nicotine content condition was unchanged, $F(4, 171) = 2.66$, $p = 0.04$. Men (n=20) and women (n=17) did not differ in the magnitude of difference in choice overall (no main effect of sex, $F(1, 171) = 2.90$, $p = 0.09$) nor as a function of nicotine content condition (no interaction of sex by nicotine content, $F(4, 171) = 1.33$, $p = 0.26$).

Regarding the moderating effects of menthol preference, there was a significant main effect of nicotine content condition, $F(4, 171) = 3.08$, $p = 0.02$, but a non-significant main effect of menthol, $F(1, 171) = 0.15$, $p = 0.70$. The lack of a significant main effect for menthol indicated similar magnitudes of difference in choice among menthol and non-menthol smokers when collapsing across all nicotine content conditions. More importantly, the interaction between menthol preference and nicotine content condition was significant—$F(4, 171) = 3.64$, $p = 0.007$—providing evidence that menthol preference moderated the relationship between nicotine condition and puff choice. In other words, the pattern of difference in puff choice across nicotine conditions

differed between menthol and non-menthol smokers. To better understand how choice varied across nicotine conditions, follow up analyses examined the effect of nicotine condition on puff choice separately for menthol and non-menthol smokers.

For non-menthol smokers (n=17), there was a significant main effect of nicotine content condition, $F(4, 77) = 5.14$, $p = 0.001$. Inspection of Figure 6 reveals an orderly dose effect in the number of NIC puff choices. The number of NIC choices for the 1.3 and 2.3 mg/g conditions were significantly lower than that for each of the higher nicotine content conditions, except for 2.3 vs. 5.5 mg/g; there were no significant differences among the three highest nicotine content conditions. NIC choices at the two lowest contents were not significantly greater than that of the VLNC, as the respective 95% confidence intervals in Figure 6 each overlap with eight.

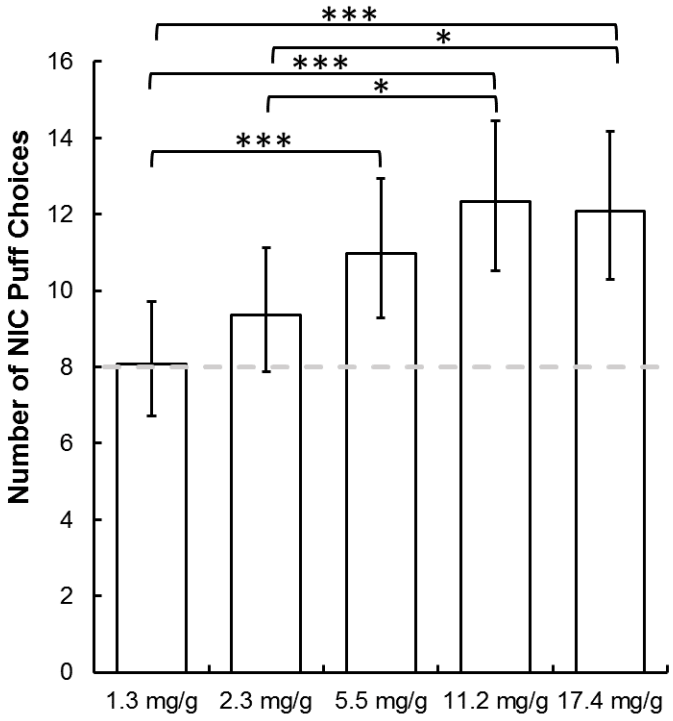

Figure 6 Mean number of NIC choices by nicotine content condition for non-menthol smokers. Vertical bars represent 95% confidence intervals. Horizontal bars indicate significant pairwise differences between nicotine content conditions. Horizontal dashed line at 8 indicates no difference in choice from VLNC. ***p<.001, *p<.05.

For menthol smokers (n=20), in sharp contrast, the main effect of nicotine content condition was not significant, $F(4, 94) = 1.06$, $p = 0.38$. As shown in Figure 7, there was a zig-zag pattern in the number of NIC choices across nicotine content conditions, with no significant differences between any conditions. The number of NIC choices was significantly greater than choices for the VLNC cigarette for 1.3, 5.5, and 17.4 mg/g, but not the intermediate contents of 2.3 or 11.2 mg/g.

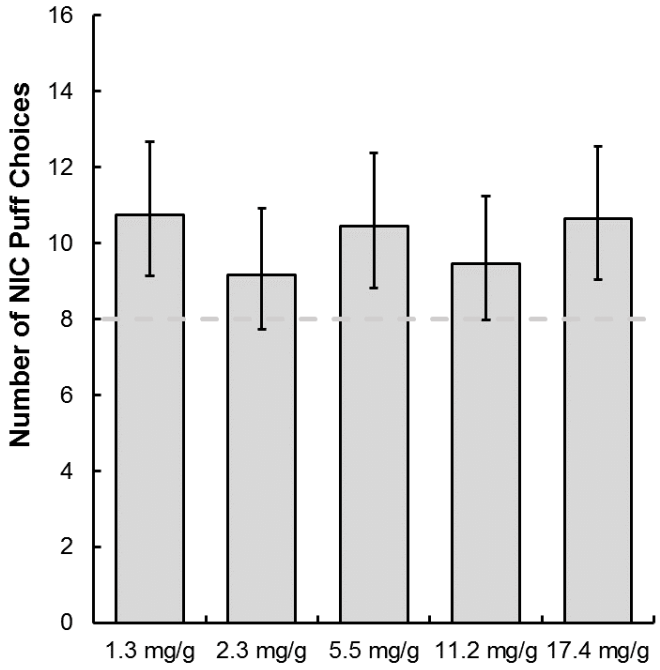

Figure 7 Mean number of NIC choices by nicotine content condition for menthol smokers. Vertical bars represent 95% confidence intervals. Horizontal dashed line at 8 indicates no difference in choice from VLNC.

In the model testing the moderating effects of ethnicity (African American/Black [n=15] vs. Caucasian [n=18]), main effects of nicotine condition and ethnicity were not significant—$F(4, 151) = 1.64$, $p = 0.17$ and $F(1, 151) = 0.24$, $p = 0.63$, respectively. However, there was a significant

interaction of nicotine condition by ethnicity, F(4, 151) = 2.66, p = 0.04. Means and 95% confidence intervals for NIC choices by nicotine condition and ethnic group are displayed in Figure 8. Analysis of each ethnicity separately revealed a significant main effect of nicotine condition for Caucasians (F(4, 81) = 4.14, p = 0.003), but not for African American/Black smokers (F(4, 70) = 0.20, p = 0.94).

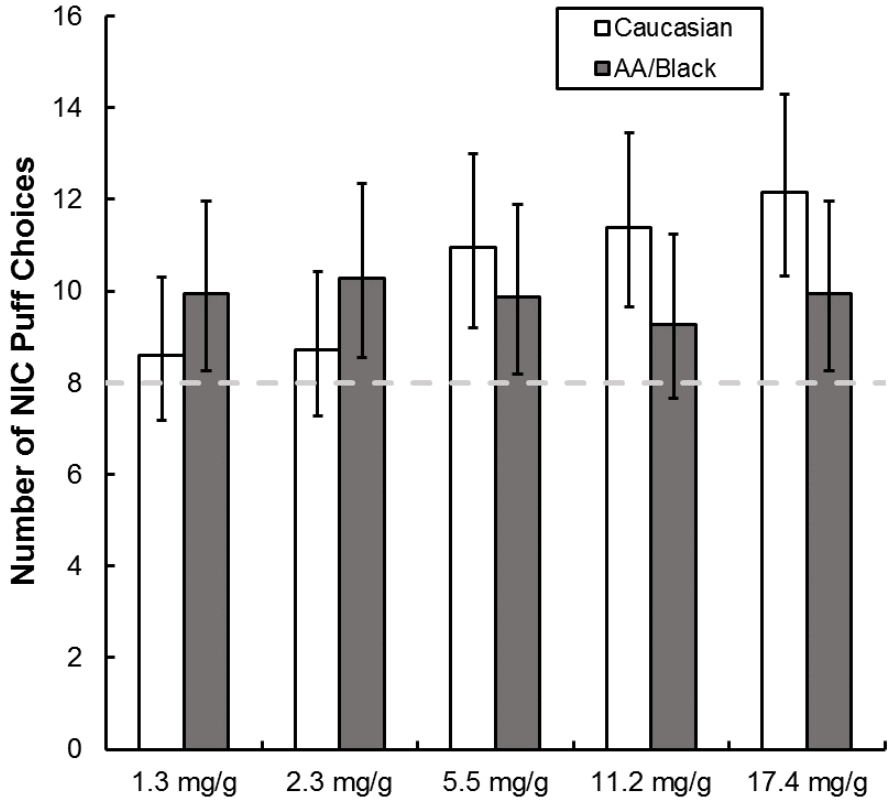

Figure 8 Mean number of NIC choices by nicotine content and ethnicity. Vertical bars represent 95% confidence intervals. Horizontal dashed line at 8 indicates no difference in choice from VLNC.

Two additional post-hoc exploratory analyses were run in an attempt to distinguish between effects due to menthol preference and ethnicity. The first model explored the moderating effect of menthol preference in Caucasian participants. A similar analysis among African

American/Black participants was not possible, as 14 out of 15 were menthol smokers (4 out of 18 Caucasians smoked menthol cigarettes), but the moderating effect of ethnicity was able to be explored among menthol smokers. In the model assessing the moderating effect of menthol in Caucasian smokers, there was a significant main effect of nicotine condition—$F(4, 76) = 3.00$, $p = 0.02$—but the main effect of menthol and the interaction of menthol by nicotine condition were not significant—$F(1, 76) = 0.06$, $p = 0.81$ and $F(4, 76) = 1.38$, $p = 0.25$, respectively. Inspection of the mean number of NIC choices by menthol preference for Caucasian smokers displayed in Figure 9, shows a dose response increase in NIC Choices among non-menthol smokers (n=14) but a zig-zag pattern across nicotine contents for menthol smokers (n=4).

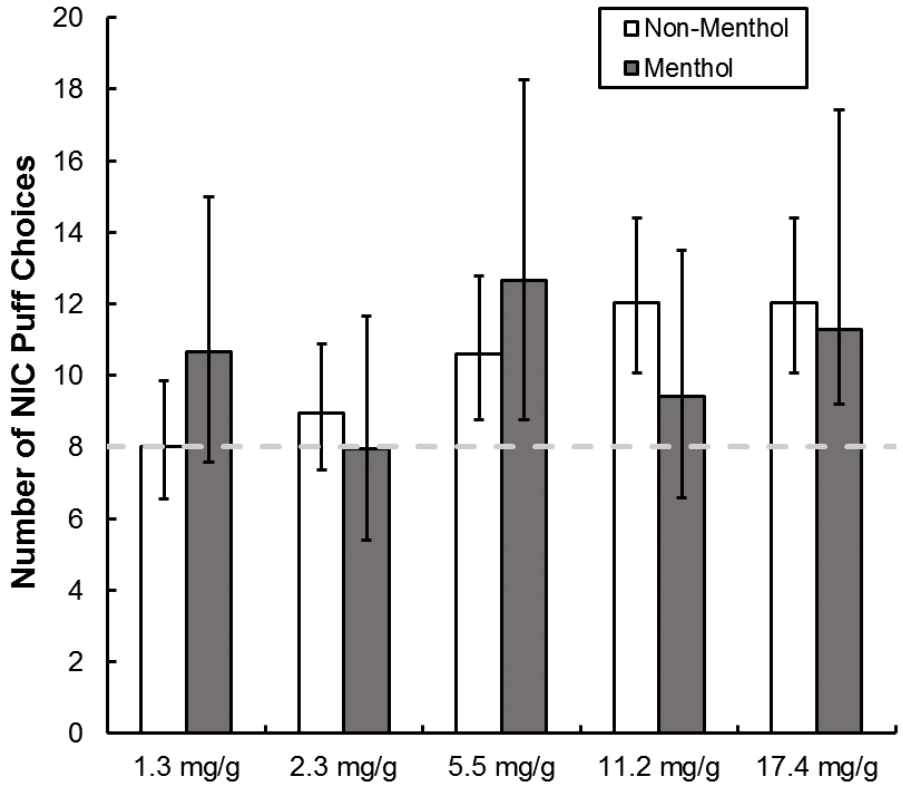

Figure 9 Mean number of NIC choices by nicotine content and menthol for Caucasian smokers. Vertical bars represent 95% confidence intervals. Horizontal dashed line at 8 indicates no difference in choice from VLNC.

In the exploratory model assessing the moderating effect of ethnicity (African American/Black [n=14] vs. Caucasian [n=4]) in menthol smokers, neither the main effect of nicotine condition nor ethnicity were significant—$F(4, 79) = 1.32$, $p = 0.27$ and $F(1, 79) = 0.29$, $p = 0.60$, respectively. The interaction between nicotine condition and ethnicity was also not significant, $F(4, 79) = 1.27$, $p = 0.29$. Means and 95% confidence intervals for NIC choices by ethnic group for menthol smokers are displayed in Figure 10.

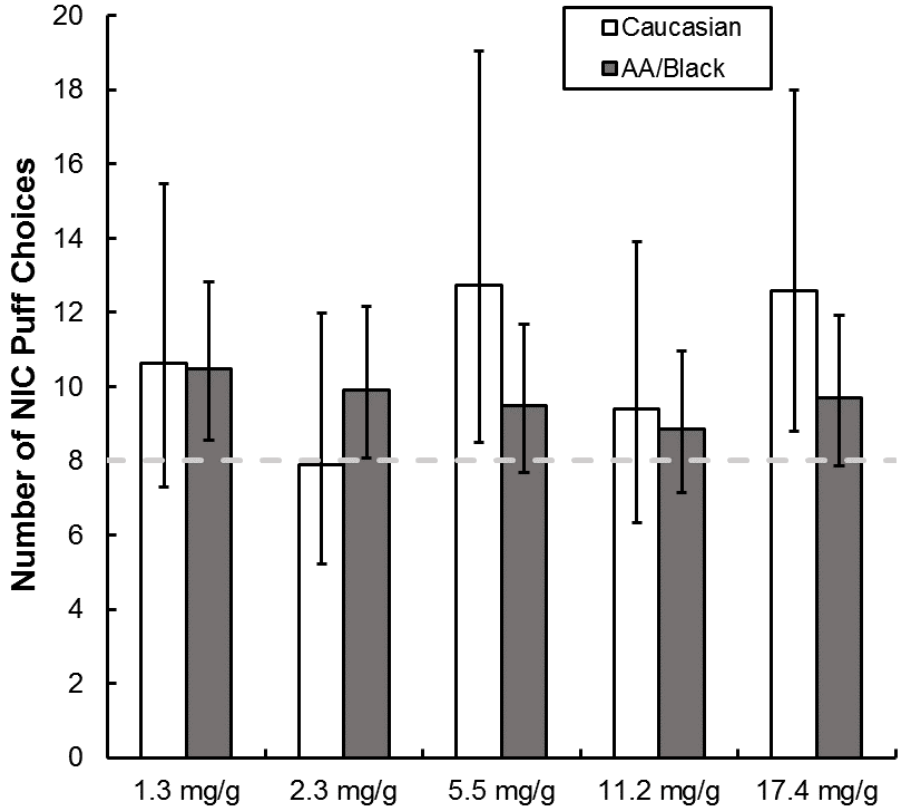

Figure 10 Mean number of NIC choices by nicotine content and ethnicity for menthol smokers. Vertical bars represent 95% confidence intervals. Horizontal dashed line at 8 indicates no difference in choice from VLNC.

Although individual cell sizes were small, this series of analyses suggest that choice across nicotine contents varied among Caucasian smokers due to menthol preference but not between ethnicities in menthol smokers. Overall, it is not clear whether differences in choice were due to ethnicity or menthol preference.

3.4 Aim 2 Analyses

3.4.1 Main effect of nicotine condition on sensory perceptions

Analysis of the composite ACP difference score (NIC – VLNC) revealed a significant main effect of nicotine condition, $F(4, 141) = 6.61$, $p = 0.00007$. Estimated mean ACP difference scores (with 95% CIs) by nicotine condition are illustrated in Figure 11. Other than the 1.3 mg/g condition, all nicotine contents were rated significantly more pleasurable than the concurrently presented VLNC, as denoted by 95% CIs not overlapping with zero. Significant between-condition differences are indicated by asterisks and brackets in Figure 11. Notably, the magnitude of difference was greater for: 17.4 mg/g vs. contents ≤ 5.5 mg/g; 11.2 mg/g vs. contents ≤ 2.3 mg/g; and 5.5 mg/g vs 1.3 m/g. This pattern of differences was consistent across almost all of the individual items comprising the composite ACP—'Liking', 'Satisfying', 'How Much Nicotine', and 'Strong'. The main effect of nicotine content was marginal for 'Flavor'. Estimated means, standard errors, and F-values for each of the sensory perceptions are presented in Table 4.

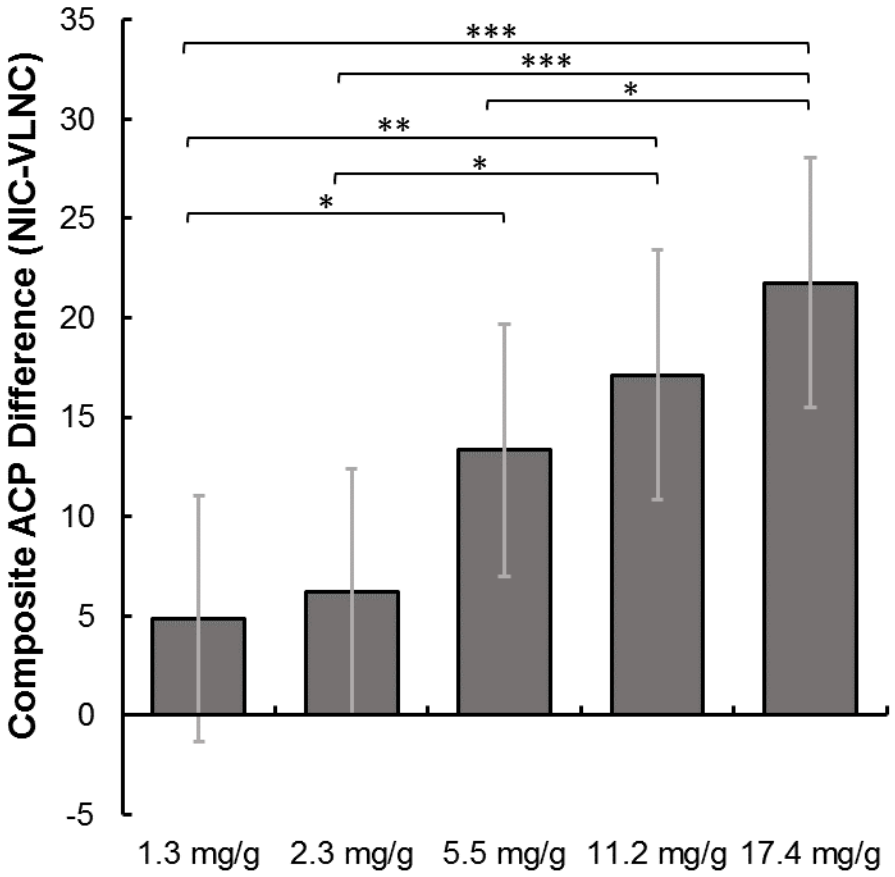

Figure 11 Mean difference (NIC-VLNC) in ACP composite score by nicotine content condition. Vertical bars represent 95% confidence intervals. Horizontal bars indicate significant pairwise differences between nicotine content conditions. ***p<.001, **p<.01, *p<.05.

Table 4 Means and standard errors for sensory response difference scores (NIC – VLNC) by nicotine condition

	Nicotine Content Condition											
	1.3 mg/g		2.3 mg/g		5.5 mg/g		11.2 mg/g		17.4 mg/g			
Variable	M	SE	M	SE	M	SE	M	SE	M	SE	F	*p*
Liking	6.82	3.56	7.66	3.56	16.24	3.65	16.40	3.61	26.97	3.61	6.16	< 0.001
Satisfying	5.22	3.59	6.08	3.59	17.42	3.68	18.30	3.64	25.30	3.64	6.45	< 0.001
How Much Nicotine	7.66	3.46	6.73	3.46	12.86	3.54	18.62	3.50	19.69	3.50	3.76	0.006
Strong	0.45	3.77	3.76	3.77	12.51	3.85	19.55	3.81	21.12	3.81	9.06	< 0.001
Flavor	4.00	3.71	6.78	3.71	8.02	3.80	12.89	3.76	15.93	3.76	2.20	0.07

Note. M is mean; SE is standard error; F(4, 141) is for main effect of nicotine content condition.

3.4.2 Exploratory moderation analyses

Given the significant moderating effects of menthol and ethnicity on the relationship between nicotine content condition and choice behavior, menthol preference and ethnicity were also examined as a potential moderators of acute sensory perceptions across nicotine contents. This series of post hoc analyses explored whether any differences in sensory perceptions existed among levels of each factor (i.e., a main effect of menthol preference or ethnicity) and whether such differences varied as a function of nicotine content condition (i.e., an interaction with nicotine content). The ACP composite variable was examined first; any significant effects involving menthol or ethnicity were followed up by examining the five individual sensory perception items.

Results of the menthol analyses by measure and effect are presented in Table 5. There was a significant interaction of menthol and nicotine condition for the ACP composite score. Among the individual sensory measures, there were significant effects (aside from significant main effects of nicotine content condition) for ‘Strong’, ‘Flavor’, and ‘How much nicotine’.

Table 5 Results of sensory perception analyses by nicotine condition, menthol, and nicotine condition by menthol

	Effect								
	Nicotine Content Condition			Menthol			Nic Content X Menthol		
Variable	F	DF	*p*	F	DF	*p*	F	DF	*p*
ACP Composite	7.51	4, 137	< 0.001	0.72	1, 35	0.40	2.66	4, 137	0.04
Liking	6.29	4, 137	< 0.001	0.0001	1, 35	0.99	1.07	4, 137	0.38
Satisfying	6.76	4, 137	< 0.001	0.07	1, 35	0.79	1.60	4, 137	0.18
How Much Nicotine	4.49	4, 137	0.002	0.25	1, 35	0.62	2.66	4, 137	0.04
Strong	9.95	4, 137	< 0.001	4.29	1, 35	0.046	1.59	4, 137	0.18
Flavor	2.88	4, 137	0.03	1.21	1, 35	0.29	4.31	4, 137	0.003

Note. DF is degrees of freedom; ACP is Acute Cigarette Perception

Menthol preference significantly moderated the relationship between nicotine condition and the difference in ACP composite sensory response, $F(1, 137) = 2.66$, $p = 0.04$. Illustrated in Figure 12, the magnitude of difference in the ACP composite score increased across contents for non-menthol smokers yet was flat for contents ≤11.2 mg/g in menthol smokers. Among non-menthol smokers, the magnitude of difference in ACP was significantly greater for the 17.4 and 11.2 mg/g contents vs. contents ≤ 5.5 mg/g; contents 17.4 and 11.2 mg/g were not significantly different. For menthol smokers, the magnitude of difference in ACP responding for the 17.4 mg/g content was significantly greater than all other contents, except 5.5 mg/g.

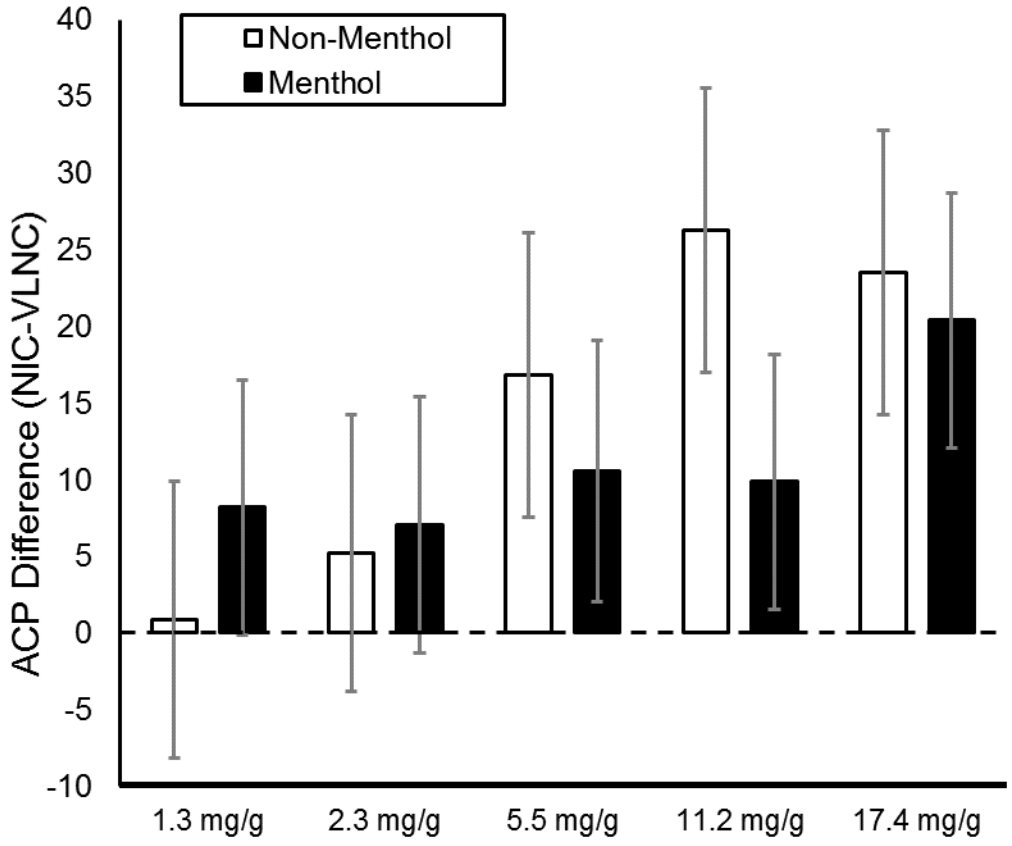

Figure 12 Mean difference (NIC-VLNC) in ACP composite response by menthol preference and nicotine content condition. Bars represent 95% confidence intervals.

There was a significant main effect of menthol preference for 'Strong', $F(1, 35) = 4.29$, $p = 0.046$. As shown in Figure 13, the magnitude of difference between the VLNC and higher nicotine content cigarette was smaller for menthol versus non-menthol smokers, across nicotine conditions. However, the interaction of menthol by nicotine content condition was not significant, $F(4, 137) = 1.59$, $p = 0.18$.

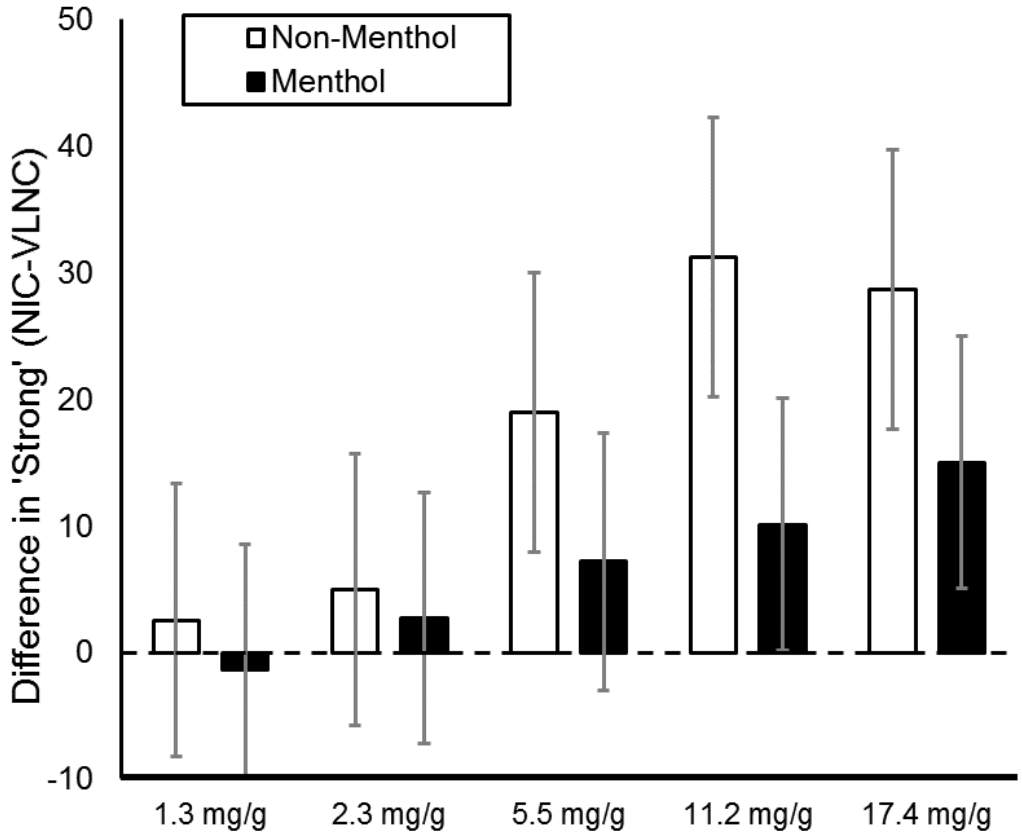

Figure 13 Mean difference (NIC-VLNC) in 'Strong' response by menthol preference and nicotine content condition. Bars represent 95% confidence intervals.

For 'Flavor', the main effect of menthol preference was not significant, $F(1, 35) = 1.21$, $p = 0.28$, but there was a significant interaction of menthol by nicotine content condition, $F(4, 137) = 4.31$, $p = 0.003$. Inspection of Figure 14 shows a steady increase in the magnitude of difference in 'Flavor' as nicotine content increases for non-menthol—but not menthol—smokers. For non-menthol smokers, contents ≥5.5 mg/g were rated as having significantly higher 'Flavor' than VLNC and the magnitude of difference was significantly greater for all contents ≥ 5.5 vs ≤ 2.3 mg/g. For menthol smokers, only contents 2.3 and 17.4 mg/g were rated as having significantly more 'Flavor' than the VLNC cigarette, with no significant pairwise differences among nicotine content pairs.

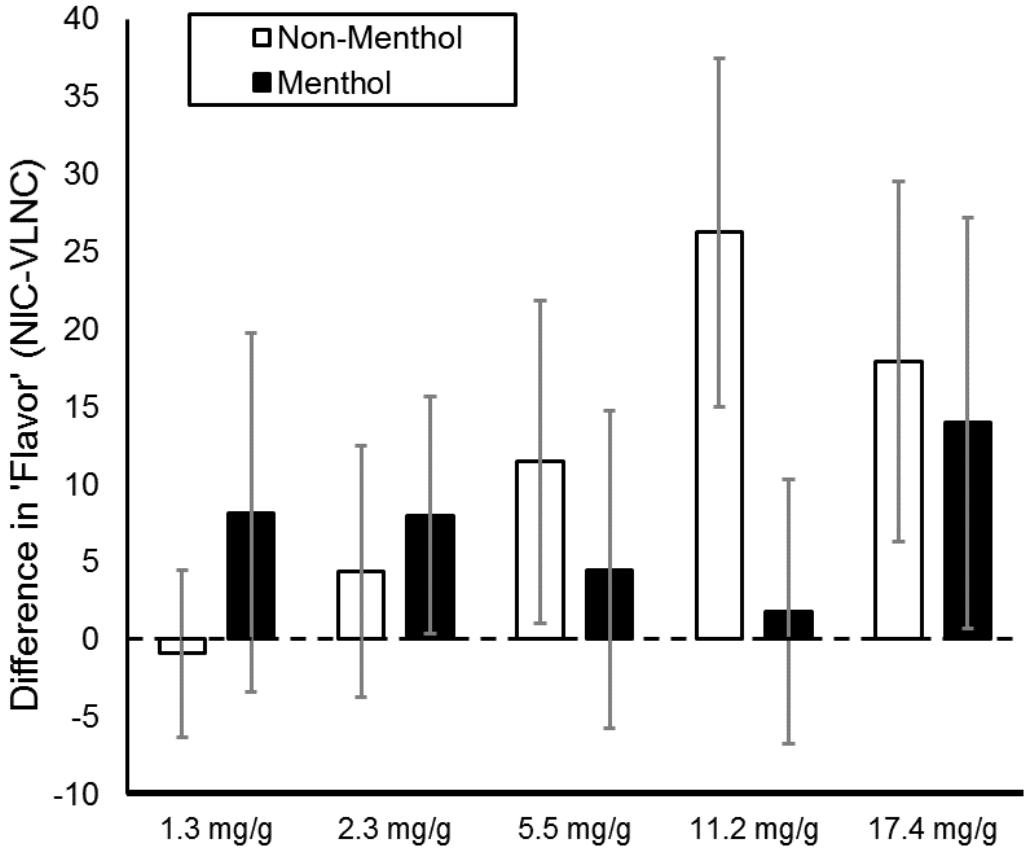

Figure 14 Mean difference (NIC-VLNC) in 'Flavor' response by menthol preference and nicotine content condition. Bars represent 95% confidence intervals.

For the analysis testing the effects of ethnicity on the relationship between the ACP composite variable and nicotine condition, there was no main effect of ethnicity, $F(1, 31) = 2.71$, $p = 0.11$, and no interaction of ethnicity and nicotine condition, $F(4, 121) = 0.95$, $p = 0.44$. Estimated mean difference in ACP by nicotine condition and ethnicity is shown in Figure 15.

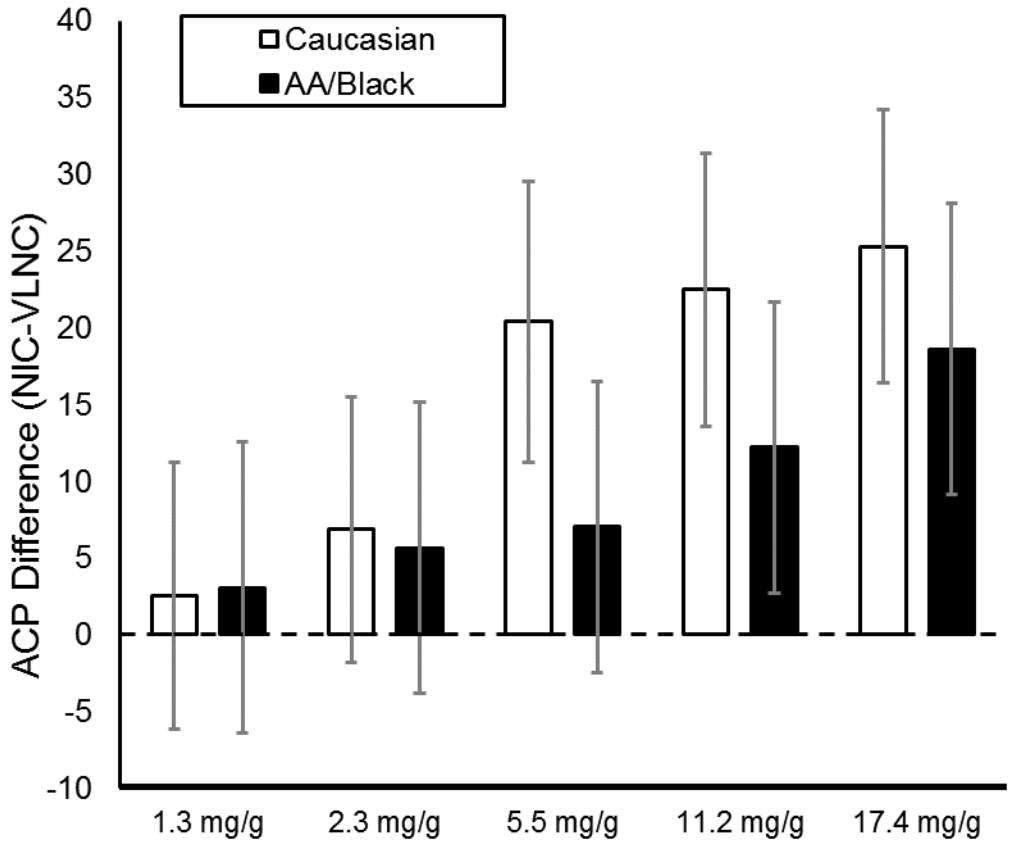

Figure 15 Mean difference (NIC-VLNC) in ACP composite response by ethnicity and nicotine content condition. Bars represent 95% confidence intervals.

3.4.3 Mediation analysis

Difference in the composite ACP measure (NIC – VLNC) was examined as a potential mechanism underlying the relationship between nicotine content condition and choice behavior using mediation analysis. Results of the mediation model are presented in Table 6. ACP composite ratings significantly mediated the relationship between nicotine content condition and puff choice—indirect effect: a*b = 0.43, 95% CI [0.21, 0.69], p = 0.0005. In sum, nicotine content condition influenced puff choice because as the nicotine content increased across conditions,

cigarettes with higher nicotine contents had increasingly larger acute positive sensory ratings (i.e., ACP) which led to more choices for that higher nicotine content cigarette.

Table 6 Results of the mediation analysis

Path	Direct and indirect effects	B	95% CI LL	95% CI UL
a	ACP on Nicotine Condition	4.48***	2.31	6.64
b	Number of NIC puffs	0.10***	0.07	0.13
c	NIC Puffs on Nicotine Condition	0.07	-0.33	0.47
a*b	Indirect effect: NIC Puffs on Nicotine Condition via ACP	0.43***	0.21	0.69

Note. B is beta; CI is confidence interval; LL is lower limit; UL is upper limit; ***p<.001, **p<.01, *p<.05.

3.4.4 Moderated mediation analysis

Moderated mediation analysis was run to determine whether menthol preference affected how the relationship between nicotine content condition and ACP composite explained puff choice. The goal of this analysis was to test the index of moderated mediation (i.e., the product of the interaction effect of menthol by nicotine content condition predicting the moderator and the effect of the moderator predicting puff choice).

Results of the moderated mediation model are presented in Table 7 and an illustration of the statistical model is presented in Figure 16 to ease interpretation. The indirect effect of ACP in the analysis of puff choice regressed on the Menthol by Nicotine Content Condition interaction was not significant, indicating that there was no moderated mediation—indirect effect: a_3*b = -0.38, 95% CI [-0.83, 0.01], $p > 0.05$. In other words, ACP's mediation of the relationship between

nicotine content condition and choice behavior did not vary between menthol and non-menthol smokers.

Table 7 Results of the moderated mediation analysis

Path	Direct and indirect effects	B	95% CI LL	95% CI UL
a_1	ACP on Nicotine Condition	6.64***	3.53	9.75
a_2	ACP on Menthol (Non-Menthol = 0, Menthol = 1)	-3.27	-11.23	4.70
a_3	ACP on Menthol x Nicotine Condition	-3.92	-8.12	0.28
b	NIC Puffs on ACP	0.10***	0.07	0.13
c	NIC Puffs on Nicotine Condition	0.07	-0.33	0.47
a_1*b	Indirect effect: NIC Puffs on Nicotine Condition via ACP	0.64***	0.32	1.03
a_3*b	Indirect effect: NIC Puffs on Menthol x Nicotine Condition via ACP	-0.38	-0.83	0.01

Note. B is beta; CI is confidence interval; LL is lower limit; UL is upper limit; ***p<.001

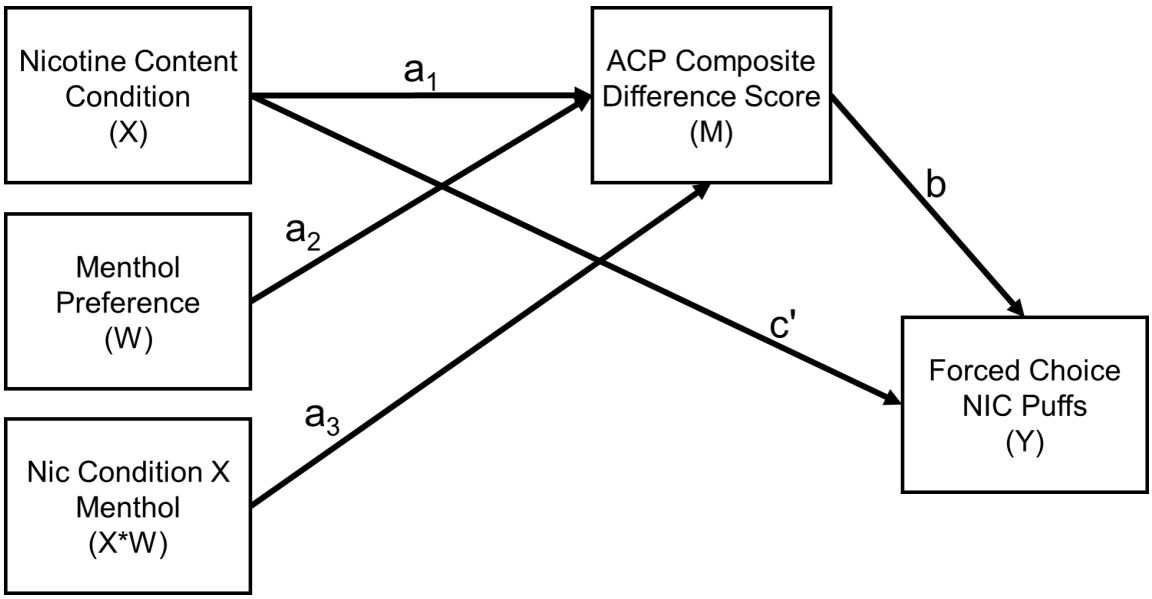

Figure 16 Illustration of the statistical model for moderated mediation analysis. Boxes represent variables entered into the model; lines represent effects, with arrows pointing towards the predicted variable. Path a_1 is the effect of the mediator (M) regressed on the independent variable (X); Path a_2 is the effect of the mediator (M) regressed on the moderator variable (W); Path a_3 is the effect of the mediator (M) regressed on the interaction of the independent and moderator variables (X*W); Path b is the effect of the dependent variable (Y) regressed on the mediator variable (M); Path c´ is the effect of the dependent variable (Y) regressed on the independent variable (X).

3.5 Aim 3 Analysis

3.5.1 Likelihood of meeting the 12 out of 16 puff criterion

This analysis sought to test how much of a nicotine reduction is needed from the 17.4 mg/g dose to see a decline in the likelihood of reliably self-administering the NIC dose significantly greater than the VLNC, using an a priori binomial distribution criterion: ≥12 NIC choices. Estimated percent of the sample choosing the higher nicotine content cigarette at each nicotine content condition and results of the analysis are presented in Table 8. Overall, there was a marginal main effect of nicotine condition on the likelihood of meeting the 12 out of 16 puff criterion, $F(4, 176) = 2.13$, $p = 0.08$.

Table 8 Results of the binominal logistic mixed model analysis

Nicotine Condition	Estimated Percent ≥12 NIC Choices		95% CI			95% CI	
		B	LL	UL	OR	LL	UL
1.3 mg/g	27.01%	-1.16	-2.30	-0.02	0.31*	0.10	0.98
2.3 mg/g	20.66%	-1.51	-2.68	-0.34	0.22**	0.07	0.71
5.5 mg/g	37.84%	-0.66	-1.78	0.46	0.52	0.17	1.59
11.2 mg/g	46.24%	-0.31	-1.42	0.79	0.73	0.24	2.21
17.4 mg/g	54.07%	—	—	—	1.00	—	—

Note. NIC is the higher nicotine content cigarette; B is beta; CI is confidence interval; LL is lower limit; UL is upper limit; OR is odds ratio; * $p < .05$, ** $p < .01$ for difference relative to 17.4 mg/g group.

Additional post hoc exploratory analyses separately assessed the moderating effects of menthol preference and ethnicity (Caucasian vs. African American/Black). For ethnicity, there were no significant effects—F's <1.52, p's > 0.20. For menthol, there was no significant main

effect of menthol preference nor a significant interaction of menthol preference by nicotine condition, $F(1,171) = 0.01$, $p = 0.94$ and $F(4, 171) = 1.61$, $p = 0.17$, respectively. The main effect of nicotine condition was significant when these additional factors were included in the model, $F(4, 171) = 2.43$, $p = 0.049$.

4.0 Discussion

The FDA has considered reducing the maximum allowable nicotine content in cigarettes to make it easier for established smokers to quit (Benowitz & Henningfield, 1994, 2013, 2018; FDA, 2018). A large-scale longitudinal clinical trial—designed to inform potential policy—identified the reduction in nicotine from a standard nicotine content (15.8 mg/g) needed to significantly reduce cigarette consumption (Donny et al., 2015). The primary aim of the current study was to validate use of a shorter—potentially more efficient—within-subjects forced-choice procedure in identifying the nicotine content reduction needed to reduce relative reinforcement (used here as an index of self-administration behavior). Successful validation of this procedure would show consistency with the primary outcome from Donny et al. (2015)—significant reductions in choice between cigarettes containing ≥5.5 mg/g and those ≤2.3 mg/g. Results from the forced-choice procedure were complemented by data on sensory perceptions of acute use of the research cigarettes to further examine mechanisms underlying changes in forced-choice behavior across nicotine content conditions.

Overall, the pattern of results from the current forced-choice procedure were not consistent with those of Donny et al. (2015). In retrospect, it seems that the different approaches (i.e., within-subjects testing of acute relative reinforcement vs. randomized between-subjects testing of smoking reduction due to extended use) were assessing divergent constructs. Rather than observing significantly greater NIC choices for all contents ≥5.5 mg/g vs. ≤2.3 mg/g (the pattern observed in Donny et al.), only the 17.4 mg/g condition had significantly greater NIC choices than both conditions ≤2.3 mg/g. Potential reasons for the lack of concordance between the current study and Donny et al. include methodological differences (e.g., within-subjects vs. between-subjects

designs, acute vs. extended use, relative vs. absolute reinforcement, etc.; see section 4.1 Strengths and Limitations) and confounding due to menthol preference and/or ethnicity (African American/Black or Caucasian).

The number of NIC choices increased linearly across nicotine conditions for non-menthol smokers but was flat across nicotine conditions for menthol smokers. Neither outcome (linear or flat across nicotine conditions) nor the presence of moderating effects of menthol (and/or ethnicity) are consistent with the pattern of results from Donny et al. (2015). Discussed in greater detail below, it is important to note that ethnic homogeneity within menthol preference groups made it unclear whether the menthol group differences in choice were actually ethnicity effects. Together, these results do not validate use of outcomes from the current within-subjects forced choice procedure to predict results of longer clinical trial research—changes in acute relative reinforcement due to differences in nicotine content in cigarettes did not align with patterns of self-administration of cigarettes differing in nicotine content over six-weeks of use.

Although the current procedure was not able to produce a pattern of results consistent with Donny et al. (2015), findings indicate that relative reinforcement was sensitive to changes in nicotine content. Overall, each higher nicotine content cigarette was chosen significantly more than the 0.4 mg/g alternative, similar to results from Higgins et al. (2017). Unlike Higgins et al., there was a threshold for relative reinforcement—choice for the 17.4 mg/g cigarette was significantly greater than contents $\leq$ 2.3 mg/g, suggesting that reducing nicotine content in cigarettes to levels $\leq$ 2.3 mg/g may diminish reinforcement from these cigarettes (relative to a conventional nicotine content cigarette).

It is unclear whether reductions in acute relative reinforcement observed in the lab would translate to fewer cigarettes smoked over longer periods of time in the real world. Results of a

recent trial by Hatsukami et al. (2018) offer initial support for potential translation. In their 20-week long study, Hatsukami and colleagues randomized smokers to one of three groups: 1) use cigarettes gradually declining in nicotine content (15.5, 11.7, 5.2 2.4, 0.4 mg/g; four weeks per content); 2) use only cigarettes containing 15.5 mg/g; or 3) use only cigarettes containing 0.4 mg/g. At the end of the study, participants in the gradual reduction group smoked a similar number of cigarettes per day as those randomized to the 15.5 mg/g control condition, but reduced cigarette consumption (to a non-significant degree) during the 2.4 and 0.4 mg/g conditions. Those randomized to exclusively use the 0.4 mg/g over the entire 20-week study smoked significantly fewer cigarettes per day than those in both the gradual reduction and control conditions. In the current study, choice of cigarettes ≤2.4 mg/g were significantly lower than choice for the 17.4 mg/g condition and all nicotine contents were chosen significantly greater than the 0.4 mg/g alternative. Taken together, policy reducing the maximum allowable nicotine content in cigarettes to 0.4 mg/g may lead to a reduction in smoking. Given the continued—albeit reduced—smoking of cigarettes ≤2.4 mg/g observed in extended use trials (Donny et al., 2011, 2015; Hatsukami et al., 2018), it is likely that smokers will continue to use cigarettes containing 2.4 mg/g despite a reduction in reinforcement from nicotine per se.

Post hoc exploratory analyses assessed differential patterns in choice across nicotine contents due to sex, menthol preference, and ethnicity. Men and women chose similarly across nicotine content conditions—sex did not significantly moderate the relationship between nicotine condition and choice behavior. This lack of sex differences was not surprising, despite the well-established sex differences in nicotine reinforcement showing that women are generally less sensitive to changes in nicotine than men (Perkins, 2008; Pogun, Yararbas, Nesil, & Kanit, 2017). This study was not originally designed nor powered to detect any between-group differences, and

so it is likely that the small sample size led to an underpowered analysis of sex differences. Additional research in larger samples would be needed to better examine sex differences in acute relative reinforcing effects of SPECTRUM cigarettes differing in nicotine content.

Menthol preference moderated choice behavior across nicotine content conditions. Looking at each of these subgroups separately, choice results for non-menthol smokers followed a linear dose response with increases in NIC choices as nicotine content also increased. However, choice behavior for menthol smokers across nicotine contents was much more erratic, with no dose response effect. Together, these results initially suggest that non-menthol smokers may have been more sensitive to changes in nicotine content of these SPECTRUM cigarettes than menthol smokers, subject to the limitations of this study procedure. However, ethnic homogeneity within menthol preference groups (Caucasian within non-menthol and African American/Black within menthol) makes it difficult to separate effects due to menthol preference vs. ethnicity.

A series of exploratory post hoc analyses attempting to parse menthol from ethnicity were largely inconclusive. As with menthol preference, there was a significant interaction between ethnicity (Caucasian vs. African American/Black) and nicotine condition. Caucasians had a mostly linear increase in choice across nicotine contents and choice among African American/Black did not have a perceptible pattern—consistent with the patterns of choice for non-menthol and menthol smokers, respectively. Follow-up analyses comparing menthol preference within Caucasians and ethnicity within menthol smokers did not clarify the situation (most likely due to underpowered analyses among small subgroups of smokers). Within Caucasian smokers, there appeared to be a linear increase in choice for non-menthol and a zig-zag pattern for menthol smokers, but none of the menthol effects were significant. Among menthol smokers, there were no differences in choice between Caucasian and African American/Black participants. In sum, it is unclear whether the

menthol effects on choice behavior were due to ethnicity. Further research in a larger sample with ethnic heterogeneity within menthol preference groups is needed to better understand whether menthol preference, ethnicity, or a combination of these factors differentially influences acute relative reinforcement from cigarettes varying in nicotine content.

Acute sensory perceptions were found to vary across the range of nicotine content conditions. Overall, there was a significant main effect of nicotine content condition for each measure of pleasurable sensory perceptions of smoking—the magnitude of difference in sensory ratings between the NIC and VLNC cigarettes increased across doses, consistent with earlier research on sensory perceptions of acute RNC cigarette use (Arger et al, 2017; Bergeria et al., 2019; Cassidy et al., 2018a, 2018b, 2019; Faulkner et al., 2017, 2018; Hatsukami et al., 2013; Streck et al., 2019). In sum, these data confirm the notion that a cigarette's nicotine level increases its immediate acute pleasurable subjective effects.

A particularly novel contribution of this research is the finding that the composite of acute subjective perceptions significantly mediated the relationship between nicotine content condition and forced-choice behavior. Increases in nicotine content led to more choices for the higher nicotine content cigarette because higher nicotine content cigarettes elicited a greater magnitude of difference in acute pleasurable subjective effects vs. the VLNC cigarette, and smokers preferred the cigarette providing more pleasurable subjective effects. This mediation process did not vary due to menthol preference, showing that ACP mediated forced-choice responding equally among menthol groups, despite menthol's blunting of sensory perceptions. The relationship between ACP and choice is consistent with previous research relating pleasurable sensory perceptions to choice of cigarettes containing 0.4 and 16-17 mg/g (Perkins, Karelitz, & Kunkle, 2018), but extend those findings to examine these associations across narrower differences in nicotine contents. Other

research in a sample of smokers "vulnerable to nicotine addiction" reported similar results, with pleasurable sensory perceptions predicting increased operant responding to earn higher nicotine content cigarettes when presented concurrently with the opportunity to also earn a lower content cigarette (Bergeria et al., 2019). Altogether, these results substantially document that acute pleasurable subjective perceptions constitute one of the mechanisms underlying relative reinforcing effects of smoking nicotine.

Unlike the indistinguishable effects of menthol and ethnicity on choice behavior, pleasurable sensory perceptions of smoking across nicotine conditions were found to differ due to menthol preference but not ethnicity. As menthol is a flavoring added to cigarettes, it is not unexpected that it would be found to alter sensory perceptions of smoking. Nicotine and menthol provide unique, qualitatively different sensory aspects when smoking, which can come to reinforce smoking behavior through associative learning mechanisms (Ahijevych & Garrett, 2010). In unmentholated cigarettes, higher levels of nicotine are associated with greater "throat hit" or "scratch", which can be pleasing to non-menthol, but not menthol smokers (Kreslake, Wayne, & Connolly, 2007; Wayne & Connolly, 2004). Menthol provides a reinforcing cooling sensation which reduces throat scratch and ameliorates smoke-related irritation (Kreslake, Wayne, & Connolly, 2007; Wayne & Connolly, 2004). In the context of the current study, mentholated cigarettes provided significantly different patterns of pleasurable perceptions of smoking compared to non-mentholated cigarettes.

There were linear increases for the difference in ACP composite (NIC – VLNC) for non-menthol smokers across nicotine conditions, indicating greater pleasurable responses as the nicotine content of the higher content cigarette increased. For menthol smokers, the difference in ACP composite was flat across contents ≤11.2 mg/g, but significantly increased for 17.4 mg/g.

This suggests that menthol may have blunted pleasurable sensory perceptions of cigarettes < 17.4 mg/g. Earlier research from our lab using a larger sample failed to find differences due to menthol preference on ACP composite ratings of cigarettes containing 0.4 and 16-17 mg/g of nicotine (Perkins, Karelitz, & Kunkle, 2018). The current menthol-related differences in ACP are due to assessing a wider range of nicotine contents; differences would not have emerged looking at only the 17.4 mg/g condition in the present study.

Menthol smokers rated the SPECTRUM cigarettes as less strong compared to non-menthol smokers across all nicotine contents, consistent with earlier research and the notion that menthol has analgesic effects (Davis, 2017; Hatsukami et al., 2013). Menthol smokers' subjective rating of 'Strong' may be related to the cigarette's menthol content (Kreslake, Wayne, & Connolly, 2007; Wayne & Connolly, 2004). In the current study, menthol contents of the SPECTRUM cigarettes ranged 0.91-2.08 mg/g, consistent with levels needed to experience a "slight menthol sensory effect" (Ai et al., 2016). In contrast, menthol contents of participants' self-reported brands are much higher (4.98-5.40 mg/cigarette), at or above levels needed for a "strong menthol effect" (i.e., 2.5-4.5 mg/g or 1.75-3.40 mg/cigarette; Ai et al., 2016). It is possible that the relatively lower menthol contents in the SPECTRUM cigarettes may have blunted 'Strong' ratings—as this can be an index of menthol flavoring for menthol smokers—but this would need to be directly confirmed in additional research.

Ratings of 'Flavor' varied between menthol and non-menthol smokers as a function of nicotine content condition—non-menthol smokers had orderly dose response increases in 'Flavor', but menthol smokers' ratings did not vary across nicotine content conditions. As with 'Strong', non-menthol and menthol smokers have disparate qualitative associations for a cigarette's 'Flavor' or 'Taste'. For non-menthol smokers, 'Flavor' or 'Taste' is related to throat impact and

astringency, whereas menthol smokers associate smoke concentration and added flavors (Kreslake, Wayne, & Connolly, 2007). Notably, the nicotine condition with the lowest menthol content (11.2 mg/g nicotine, 0.91 mg/g menthol) was not chosen significantly greater than the VLNC and had the smallest magnitude of difference in 'Flavor', relative to the VLNC cigarette (0.4 mg/g nicotine, 1.08 mg/g menthol). Earlier research assessing menthol smokers' responses to non-mentholated cigarettes found that menthol smokers perceive lower levels of 'Flavor' in non-menthol vs. menthol cigarettes when all other cigarette-related factors were completely matched (Strasser et al., 2013). It is possible that menthol smokers' lower ratings of 'Flavor' across nicotine contents in the current study could have been due to changes in menthol content, consistent with Strasser et al. (2013). Because menthol and nicotine contents were not manipulated independently in the present study, it is not possible to separate effects due to changes in just one of these factors.

Beyond possible cigarette-level explanations, individual differences may have also contributed to the observed variability between menthol and non-menthol preferring groups. Menthol smokers have been shown to have nicotine metabolite ratios consistent with slower nicotine clearance rates, which extends nicotine exposure from each cigarette (Alsharari et al., 2015; DeVito et al., 2016; Fagan et al., 2016). Unfortunately, nicotine metabolite ratios were not measured in the current study and earlier studies assessing the effect of nicotine metabolite ratio on smoking reduced nicotine content cigarettes were exclusively of non-menthol smokers (Bandiera et al., 2015; Faulkner et al., 2017; Mercincavage at el., 2018). Additional research may be needed to collectively assess the effects of nicotine content, menthol preference, ethnicity, and nicotine metabolite ratio on relative reinforcement of nicotine via tobacco smoke.

4.1 Strengths and Limitations

Results of the study must be interpreted within the context of the strengths and weaknesses of its design. The methods and measures of self-administration were different between the current study and Donny et al. (2015). Whereas Donny et al. (2015) assessed between-subjects effects on daily smoking self-administration (i.e. absolute reinforcing effects) of each nicotine content condition, the present study tested within-subjects effects of preference (relative reinforcement) between each content vs. the 0.4 mg/g VLNC. Use of a within-subjects design allowed for a smaller sample size, as each participant acted as their own control increasing statistical power (Cohen, 1988). Further, Donny et al. (2015) studied smokers in their natural environment over weeks of use; this type of ecological validity was lost in the lab-based study of acute self-administration. Likely due to these methodological differences, the pattern of results in acute relative reinforcement from the current study were not consistent with the earlier longitudinal dose-response effects on absolute reinforcement.

The final sample size (n=37) was smaller than what was originally proposed (and committee-approved, n=40). Although there was a significant main effect for the primary analysis of choice behavior across nicotine conditions, many of the exploratory subgroup analyses were underpowered and small cell sizes prevented further probing from yielding useful results. In other words, interesting results emerged (e.g., menthol preference moderating choice across nicotine content conditions), but the small sample size prevented further exploration to better understand the outcomes and implications (e.g., inability to distinguish menthol preference effects from ethnicity effects). Future research intending to examine differences among subgroups of smokers will need to have much larger sample sizes than what was originally proposed for the current study.

There was not enough time between each 4-puff smoking trial for complete clearance of the nicotine consumed in prior trials (the elimination half-life of nicotine in humans is about 2 hrs; Benowitz, 1988, 1996). The fixed order of the exposure trials (VLNC, NIC, NIC, VLNC) was designed to minimize carryover for the initial sampling of each cigarette while also providing adequate exposure to each to inform subsequent choice. Prior research has shown that differences between cigarettes varying in nicotine content are enhanced when compared concurrently versus independent testing sessions (Perkins et al., 2002). Concurrent presentation of cigarettes within each session of the current study likely increased sensitivity to differences between each pair, rather than attenuate such differences as would be expected had carryover effects occurred. Similarly, analyses showed no effects of trial on measures, further discounting possible carryover effects.

The choice options did not include a true placebo cigarette (i.e., 0 mg/g) for comparison, requiring all higher nicotine content cigarettes to be compared with one containing some, if minimal (0.4 mg/g), nicotine. This is different from earlier nicotine self-administration studies using other methods of delivery (i.e., IV infusion, nasal spray, transdermal patch), which are not limited to non-zero nicotine comparison levels. Smoking reduced nicotine content or "denicotinized" cigarettes (i.e., Quest 3 with 1.0 mg/g nicotine content) results in "substantial" nicotinic receptor occupancy, but at rates lower than a cigarette containing a moderate level of nicotine (Quest 1, 12.5 mg/g; Brody et al., 2009; FDA, 2018). Thus, RNC cigarettes can still deliver nicotine to the user's brain, which may combine with non-nicotine (i.e., secondary) reinforcing effects to promote use of these products (Rose, Salley, Behm, Bates, & Westman, 2010). Consistent with this idea, studies have shown that smokers will continue to smoke RNC cigarettes over extended periods of time (Donny et al., 2006, 2009, 2015; Hatsukami et al., 2018;

Smith et al., 2019), suggesting that these cigarettes are still able to reinforce smoking behavior. Taken together, nicotine and non-nicotine factors of smoking the VLNC comparison cigarette may have provided some degree of reinforcement to participants in the current study, meaning that comparisons between the VLNC and higher nicotine content cigarettes did not isolate the reinforcing effects of nicotine per se. However, 0.4 mg/g is the lowest technically feasible nicotine content for RNC cigarettes currently available (World Health Organization, 2015) and thus the lowest comparison cigarette possible.

The total number of puffs per each 2.5- to 3-hour session (16 exposure puffs and 16 forced choice puffs; 32 total puffs or ~3 full cigarettes) were generally consistent with the typical smoker who smokes multiple cigarettes in the hours after waking in the morning (Allen, Mooney, Chakraborty, & Allen, 2009; Mooney, Green, & Hatsukami, 2006). Most of the cigarettes contained low levels of nicotine, further limiting the likelihood of nicotine satiation, let alone toxicity. Half of the 16 exposure puffs were from the 0.4 mg/g VLNC cigarette and the remaining puffs during the choice trials were chosen by the participant, limiting total nicotine exposure by design. Moreover, no significant effect of trial, as mentioned previously, further rules out evidence of satiation or toxicity.

Requiring participants to rate each cigarette immediately following each exposure trial may have influenced later choice behavior due to a phenomenon known as “verbal overshadowing”. Verbal overshadowing occurs when using words to describe an inherently internal non-verbal experience—the verbal representation may not accurately reflect what was experienced (Creswell, Sayette, Schooler, Wright, & Pacilio, 2018; Schooler, 2002). Non-verbal assessment of sensory perceptions (such as the visceral dynamometer used in Creswell et al. [2018, 2019]) could be used

in future research to assess whether verbal, non-verbal, or no assessment of perceptions of acute smoking affect subsequent choice behavior.

Participants engaged in a 15-min computer task unrelated to the current study's aims after three of the four exposure trials (but none occurred once forced-choice trials began). It is unlikely that working on the computer task between exposure trials affected choice behavior during the subsequent choice trials. Nevertheless, there is no way to know whether this additional unrelated task influenced outcomes in the current study. It would be ideal for future studies aiming to assess changes in acute relative reinforcement due to differences in nicotine content to not include tasks unrelated to the main research question.

Finally, the influence of non-conscious motivations underlying choice behavior was not assessed. Broadly, cognitive factors outside of conscious awareness can influence behavior and have been incorporated into many theories on drug dependence and abuse (e.g., Baker et al., 2004; Robinson & Berridge, 1993; Tiffany, 1990). By design, the forced-choice paradigm eliminated the participant's need to decide on whether to take a puff, they only needed to choose which cigarette to take a puff from (which was found to be mediated by participants' conscious assessment of the cigarette's immediate acute pleasurable subjective effects). Non-conscious motivations would be more likely to influence choice in a free-choice paradigm where participants have the option to abstain and would need to be taken into consideration when designing such studies (e.g., Higgins et al., 2017).

4.2 Implications and Future Directions

Despite its general lack of predictive validity in comparison to the Donny et al. (2015) trial results, results of the current study may still have useful implications for informing other research intended to address nicotine reduction policy. The forced-choice procedure was sensitive to differences in relative-reinforcement due to changes in nicotine content in cigarettes, resulting in reduced relative reinforcement for contents ≤2.4 vs 17.4 mg/g. However, each higher nicotine content cigarette was chosen significantly more than the 0.4 mg/g VLNC alternative, suggesting that setting 0.4 mg/g as the maximum allowable nicotine content could lead to the greatest reduction in nicotine reinforcement overall.

The forced choice procedure may be used in future research of novel nicotine delivery methods—assessing changes in acute relative reinforcement due to changes in nicotine content per se among different versions of the same product (e.g., electronic cigarettes, heat-not-burn tobacco products, etc.). Use of this procedure may also be extended to testing differences in choice due to nicotine content per se in approved NRT medications (e.g., inhaler, gum, lozenge, etc.) as well as novel products of unknown abuse liability (e.g., "Voke" a "vaporless valve technology inhaler"; https://www.voke.com/).

Finally, the forced choice procedure may be used to assess changes in preference of nicotine products following application of novel cessation interventions or pre-treatment with novel medications. This approach could be used to initially test cross-species translation of preclinical self-administration research of changes in nicotine reinforcement following pre-treatment with a novel medication. Although this approach may have limited clinical implications, it could provide initial information on the efficacy of those prospective interventions for reducing reinforcement from nicotine.

Appendix A Cigarette Sensory Rating Form

PLEASE RATE THE CIGARETTE YOU JUST SMOKED USING THE SCALE BELOW

NOT AT ALL	VERY LITTLE	SOMEWHAT	MODERATE	VERY MUCH

1. How much did you LIKE the cigarette you just smoked?

|----|----|----|----|----|----|----|----|----|----|

2. How SATISFYING was the cigarette you just smoked?

|----|----|----|----|----|----|----|----|----|----|

3. How much NICOTINE do you think that cigarette contained?

|----|----|----|----|----|----|----|----|----|----|

4. How STRONG was that cigarette?

|----|----|----|----|----|----|----|----|----|----|

5. How HARSH was that cigarette?

|----|----|----|----|----|----|----|----|----|----|

6. How much FLAVOR was in that cigarette?

|----|----|----|----|----|----|----|----|----|----|

7. How SMOOTH was that cigarette?

|----|----|----|----|----|----|----|----|----|----|

8. How SIMILAR TO YOUR OWN BRAND was that cigarette?

|----|----|----|----|----|----|----|----|----|----|

YOU RECEIVED CIGARETTE

A

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