Supplement Article

# Research on Behavioral Discrimination of Nicotine May Inform FDA Policy on Setting a Maximum Nicotine Content in Cigarettes

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## Abstract

**Introduction:** The Food and Drug Administration may set a maximum nicotine content in cigarettes to minimize smoking's addictiveness. Our recent research may indirectly support setting levels applicable to the population of dependent smokers below 1 mg/g (mg nicotine/g of tobacco filler).

**Methods:** Using a within-subjects design in laboratory-based studies totaling 61 nontreatment seeking adult dependent smokers, Spectrum research cigarettes with nicotine contents ranging from 1.3 to 17 mg/g (just one per session) were compared with the lowest content available, 0.4 mg/g. Identified for each participant was the smallest difference in nicotine content, or "threshold," between cigarettes that still supported behavioral discrimination (ie, ability to objectively distinguish their difference). The next lower nicotine content cigarette, not discriminated (by definition), was labeled their "subthreshold." Subjective perceptions and choice behavior were also assessed.

**Results:** Thresholds varied widely among all 61 smokers but, importantly, fewer than 7% of smokers could discriminate the two lowest, 1.3 versus 0.4 mg/g nicotine, meaning more than 90% could not do so. Moreover, we found a consistent association between their nicotine discrimination threshold and their subjective perceptions and subsequent reinforcement behavior later in the session. Specifically, a participant's discrimination threshold cigarette was also more highly rated and preferred (ie, self-administered), whereas their subthreshold cigarette was rated similarly to the 0.4 mg/g and not preferred.

**Conclusions:** Cigarette nicotine content below the threshold for perceiving nicotine's effects (ie, its discriminability) in nearly all smokers from a no nicotine comparison is likely below 1.0 mg/g, or less than or equal to 10% of that in typical commercial cigarettes.

**Implications:** Cigarettes with nicotine contents *able* to be discriminated (threshold) are also reinforcing, and those *unable* to be discriminated are **not** reinforcing, as anticipated. Yet, research explicitly comparing cigarettes with contents below 1.0 mg/g versus no nicotine (ie, a "placebo") is needed with larger samples. Results may confirm what nicotine content lower than 1.0 mg/g is below the threshold for discrimination (and self-administration) in the vast majority (>95%) of adult dependent smokers as well as teens beginning to smoke. Identifying that content would strongly support the Food and Drug Administration policy to establish a maximum nicotine content in cigarettes that will not maintain dependence.

## Introduction

In March 2018, the Food and Drug Administration (FDA) posted an Advance Notice of Proposed Rulemaking (ANPRM) to consider setting a maximum nicotine content in cigarettes that would minimize their addictiveness.[1] Anticipated consequences of such a policy include helping nicotine-dependent combustible cigarette smokers to permanently quit smoking and minimizing risks of nicotine dependence onset among the young who initiate tobacco smoking.[2] For example, as outlined in a recent simulation model,[3] availability of only cigarettes with nicotine levels below that necessary to support addiction would be expected to encourage 15%–20% of current smokers to quit each year and prevent 50% of teens from starting to smoke, thus driving down the smoking prevalence rate below 4% within 10 years, compared with 16% in 2018 (median estimates). The ultimate public health benefit of setting a "non-addictive" level of nicotine for cigarettes would be averting 4 million tobacco-related deaths over the next 50 years.[3]

This ANPRM expressly stated the FDA was particularly interested in research and comments about very low nicotine levels, consistent with interests reported by the World Health Organization,[4] but very little controlled research to date has provided clear data by which to establish what that maximum nicotine level should be. Ultimately, a variety of studies from disparate research topics may be needed to confirm a nicotine content level in smoked tobacco that does not support a persistent pattern of use promoting addiction.[5] Among these studies may be clinical trials with smokers randomized to very low nicotine cigarette use (only), to assess decline in ad-lib self-administration.[6] That type of research requires substantial investments in time and study cost to assess responses in large samples of participants over weeks and months of duration of use with these cigarettes. However, that approach may be efficiently informed by acute tests to identify the smallest difference in nicotine content of cigarettes that can be objectively perceived by the smoker. This perception can also be described as the threshold for ability to behaviorally discriminate that difference in nicotine content (ie, objectively detect the difference in psychoactive effects between cigarettes). The rationale here is that, because the psychoactive effects of nicotine are necessary to maintain dependence on tobacco smoking,[7,8] having access solely to an extremely low nicotine cigarette that smokers could not discriminate from one with virtually no nicotine is unlikely to foster dependent use of those cigarettes.[2,9]

Formal drug discrimination studies have been conducted for decades in clinical and preclinical models to objectively assess a variety of drug effects. As detailed elsewhere,[10] this research includes determining whether humans and nonhumans reliably perceive psychoactive differences between a drug versus placebo, between doses of the same drug, or between different drugs or drug combinations, based on resulting acute interoceptive (ie, central nervous system) stimulus effects, or how drug intake is perceived in the brain. Discrimination research can also help determine neural sites of a drug's action (via pretreatment with selective agonists or antagonists).[10] Although preclinical discrimination research with nicotine has a long history,[11] studies of nicotine discrimination in humans have, until recently, been limited and conducted with nonsmoked methods of rapid nicotine administration because of difficulties tightly controlling acute nicotine dosing via smoke inhalation.[12] Only now, with the recent availability of Spectrum research cigarettes (Clarence NY; http://www.xxiicentury.com/), is it possible to carefully study nicotine discrimination in humans via combustible tobacco cigarettes. Spectrum cigarettes contain different versions with well-controlled

nicotine contents across very low-to-moderate levels ranging from 0.4 to 17 mg/g (mg nicotine per g of tobacco filler).[13]

We recently conducted studies[14–16] to identify median thresholds for behavioral discrimination of smoked nicotine, for the first time to our knowledge, using Spectrum research cigarettes obtained from NIDA. For example, median threshold was somewhat higher in menthol versus nonmenthol smokers but, more importantly for this report, the number with lower thresholds was similar.[15] (We also found no effect of menthol content on subjective and choice responses to these Spectrum cigarettes.[15,17]) The specific focus in this article is to evaluate results from this research that may address the FDA's stated interest in setting a possible maximum nicotine content in cigarettes for reducing risks of nicotine dependence. A maximum nicotine content below the threshold for discrimination among the vast majority of adult dependent smokers (ie, not the median), and thus perceived as no different from a cigarette with virtually no nicotine, would be expected to also be below that necessary to maintain nicotine reinforcement and dependence.

## Methods

### Participants

Participants eligible for this research were adult smokers meeting *DSM-V* criteria for nicotine dependence,[18] healthy, and not intending to quit in the next 6 months (ie, nontreatment seekers). Exclusion criteria were current or history of dependence on other drugs, regular use of nicotine products other than cigarettes, and current treatment for serious medical or psychological problems (eg, cancer, heart disease, psychosis, major depression). A total of 77 eligible participants were enrolled in the studies using our final testing procedures,[14–16] with 61 (34 M, 27 F) completing all sessions required to confirm nicotine discrimination threshold content. The other 16 dropped out before a threshold (or confirmation of inability to discriminate) was determined. Mean (SD) characteristics of completed participants were age of 34.9 (11.0) years, typical daily smoking of 16.4 (5.9) cigarettes, and 66% preferring menthol. All also completed the Fagerstrom Test for Nicotine Dependence (FTND),[19] mean (SD) of 5.1 (1.7), so we could relate a measure of dependence severity to nicotine discrimination threshold. Racial/ethnic representation was mostly non-Hispanic white (59%), with the remainder African American (30%), more than one race (6%), or Hispanic white (5%). No differences because of sex or ethnicity were found on any of these smoking characteristics.

### Research Cigarettes

Spectrum investigational research cigarettes, manufactured by 22nd Century Group (Clarence NY; http://www.xxiicentury.com/), were obtained from NIDA's Drug Supply Program. Nicotine contents of these cigarettes started at 0.4 mg/g, the lowest available and at least 95% below typical commercial brands.[4] In each session, the 0.4 mg/g was compared with one of the higher nicotine content cigarettes, those with 1.3, 2.3, 5.3, 11.5, and 17 mg/g, with all contents comparable between menthol versus non-menthol versions. (These levels average the contents documented by manufacturers of two Spectrum batches sent in 2014 and 2015; non-menthol NRC codes were 102, 200, 300, 400, 500, and 600, and menthol NRC codes were 103, 201, 301, 401, 501, and 601). All these Spectrum cigarettes had about 9–10 mg "tar," generally similar to commercial brands.[20] (The 0.4 mg/g cigarette here is labeled "ultra-low" to differentiate it from the higher nicotine Spectrum cigarettes and

from a zero-nicotine tobacco cigarette, or true placebo, which is not available.) Because of the manufacturing process, these cigarettes do not allow smoking "compensation,"[21] or increased intensity or frequency of smoke inhalation to extract more nicotine, unlike commercial cigarettes manipulating nicotine intake via ventilated wrapping paper.[22] To compare with commercial brands more typically described by "yield" (as determined by FTC method estimating the inhaled portions of nicotine), these Spectrum research cigarettes correspond to approximately 0.03, 0.07, 0.12, 0.26, 0.7, and 0.9 mg nicotine yields (reported in http://grants.nih.gov/grants/guide/notice-files/NOT-DA-14-004.html), in comparison with the average 0.9 mg yield in typical commercial brands.[8]

## Procedures

We initially developed a procedure for assessing ability to discriminate nicotine via tobacco cigarettes[23] and then evaluated the procedure in a preliminary examination of nicotine discrimination threshold testing.[14] As described there,[14] final discrimination procedures involved separate sessions comparing the ultra-low content cigarette, 0.4 mg/g, with each of the higher nicotine content cigarettes, 1.3–17 mg/g, one per session. These procedures were adapted from behavioral discrimination research in humans with other drugs[24,25] and our earlier research on discrimination of nicotine via nasal spray.[12] Also assessed for each cigarette smoked in each session were ratings on the Acute Cigarette Perception (ACP) scale,[17] a brief self-reported measure of subjective perceptions of the cigarette just smoked, similar to perceptions often related to drug discrimination behavior.[16,25] The ACP consists of five items asking how much "nicotine," "liking," and "flavor" were experienced, and how "satisfying" and "strong" the cigarette was, with each item rated on a 0–100 visual analog scale, anchored by "not at all" to "very much." Aside from the ACP, a separate rating obtained here assessed how "similar to own brand" the cigarette was. Finally, after completing all discrimination trials, a "choice" test as to which cigarette they preferred to smoke (ie, self-administer) ended the session.[17] Details of these procedures are described later.

Here, we report the frequency of nicotine discrimination thresholds for all participants at the Spectrum cigarette nicotine content levels tested, identifying the distribution of ability to discriminate the higher content cigarettes from the lowest, the 0.4 mg/g ultra-low. The specific objective was to identify a single Spectrum nicotine content level below the discrimination threshold for the vast majority of these participants (eg, >90%), as that level could inform future research explicitly aimed at establishing a maximum content below the nicotine level that generally supports persistent cigarette smoking behavior in virtually all dependent smokers. This research was approved by the University of Pittsburgh Institutional Review Board.

### Discrimination Testing

Subjects initially learned the discrimination procedure by being tested on ability to discriminate the two most widely differing Spectrum cigarettes, 17 mg/g versus 0.4 mg/g ultra-low. Those able to discriminate then proceeded to nearly identical procedures over successive sessions comparing the 0.4 mg/g ultra-low versus either: progressively lower nicotine content cigarettes below 17 mg/g (descending order), or the 1.3 mg/g and progressively higher content cigarettes (ascending order). Testing sessions stopped when the lowest content discriminable versus the 0.4 mg/g ultra-low was identified. (This randomization to descending versus ascending order was intended to

examine whether order influenced threshold, but that was found not to be the case in the individual studies[14–16] and is not discussed here further.) Any session in which they failed to discriminate the two cigarettes being compared was again repeated at the subsequent session to confirm inability and, if again unable, that nicotine content cigarette unable to be discriminated from the 0.4 mg/g ultra-low was designated their "subthreshold." However, if able to discriminate it upon retesting, they continued in the study and were tested over successive sessions on progressively lower nicotine content cigarettes versus the 0.4 mg/g ultra-low until a subthreshold was determined. The final session involved repeat testing of the next higher nicotine content cigarette versus 0.4 mg/g, which they previously discriminated, to verify reliability of that ability to discriminate, and content of that cigarette was then designated their "threshold." If inability to discriminate occurred in the first session and that inability was repeated in the second session, study participation ended but those participant data were included in the analyses so that results on thresholds were not biased by excluding smokers who may require even higher nicotine content Spectrum cigarettes than were available to discriminate nicotine from the 0.4 mg/g ultra-low.

Particularly relevant for this report on identifying the lowest observed threshold, the few participants who discriminated the two lowest nicotine content cigarettes, 1.3 mg/g from the 0.4 mg/g ultra-low, continued on to a subsequent session testing for ability to "discriminate" between two 0.4 mg/g ultra-low cigarettes, again identified by different letter codes for participant use in identifying each. This procedure was intended to confirm that successful discrimination of the 1.3 mg/g versus 0.4 mg/g was not because of chance or some other non-nicotine content factor by which these cigarettes could be discriminated, as one 0.4 mg/g cigarette could not possibly be discriminated from another 0.4 mg/g based on their ultra-low nicotine contents, confirmed by the fact that none did, as expected. As with other participants, the final session repeated the test of discrimination between 1.3 mg/g and 0. 4 mg/g to verify ability to discriminate, establishing that content as their threshold.

Specific procedures were nearly identical for each session. Participants abstained from smoking overnight (>12 h) before each session, confirmed by CO less than or equal to 10 ppm[26] assessed by BreathCO CO monitor (Vitalograph, Lenexa, KS). In the final discrimination testing procedure used with each of these participants, the first part of each session involved them being "trained" to discriminate the designated nicotine content cigarette from the 0.4 mg/g ultra-low over four trials (two per cigarette), each cigarette being identified in each session by letter code ("A" or "B" in session 1, "C" vs. "D" in session 2, etc.). So that all would maintain motivation to learn this discrimination, they were told each correct cigarette identification during the second part of each session, the subsequent six discrimination testing trials, would result in $1 being added to their total participant payment. The six testing trials following the four training trials "tested" their ability to discriminate between the cigarettes by correctly identifying the two cigarettes by their letter code. After the last puff in each training or testing trial, subjects completed the brief self-report measure on their subjective perceptions of the cigarette. Successful discrimination was defined a priori by greater than 80% correct identifications of the two cigarettes, requiring at least five correct out of six testing trials, based on the rate of dichotomous drug-appropriate responding criterion commonly used in human drug discrimination research.[27,28]

The two cigarettes being compared per session were presented individually in random order over the four training trials and six testing trials, with 15 minutes between trials. Smoke intake from all

cigarettes was standardized at four puffs per trial, with one puff every 30 seconds and a new cigarette on each trial. This exposure is the same as in our prior nicotine discrimination testing via smoking[14,23] and as in the only other human tests on discriminating a drug inhaled by smoking, that with marijuana.[29] Smoking four puffs is also typical of exposure at the initiation of a smoker's expectations about a cigarette, including those related to reinforcement or other responses.[17,30,31] The 2-second breath "hold" duration standardized smoke intake at approximately 60 ml per puff to simulate that from most ad-lib puffing.[32] To ensure the same controlled smoke exposure from each cigarette, timing and duration of each puff was controlled by computer-displayed instructions.[33] This pattern of exposure allowed intervals of 15 minutes between trials while minimizing smoking satiation or toxicity. Total exposure over each 2-1/2 hour discrimination testing procedure was 40 puffs, or about four full cigarettes, under deprived conditions after overnight abstinence, with 20 puffs from the ultra-low and 20 from the other Spectrum cigarettes, nearly all of which were lower in nicotine than commercial brands. Therefore, total smoke exposure was comparable to, and nicotine intake probably less than, that during ad lib smoking of dependent smokers over morning hours following overnight abstinence.[34]

### Choice Procedure

After completing discrimination testing, two choice trials[35] over the remaining 30 minutes completed each session. This cigarette puff "choice" procedure assessed the relative reinforcing effects of the two cigarettes differing in nicotine contents administered during the preceding discrimination trials, similar to prior studies of factors associated with nicotine choice.[14,17,35] For each choice trial, 15 minutes apart, subjects were given simultaneously both the 0.4 mg/g ultra-low and the designated higher nicotine content cigarette, with each of them now identified again by the letter code used earlier during the training trials. They were told to smoke four puffs from some combination of the cigarettes presented (eg, some mix of the two, or all four from one or from the other), based solely on their preference. Out of 8 total puffs in the two-choice trials, the number of times the higher nicotine Spectrum cigarette was chosen relative to the 0.4 mg/g ultra-low was the measure of nicotine's relative reinforcement (with 4.0, or 50% of choices, being "chance").

### Data Analysis

Nicotine thresholds were coded as an ordinal variable for analyses, given the nonparametric intervals between Spectrum nicotine content cigarettes comprising those thresholds. The ordinal threshold variable ranged from 1 to 7, with 1 assigned to 0.4 mg/g, 2–6 assigned to those with a threshold of 1.3, 2.3, 5.3, 11.5, and 17 mg/g, respectively, whereas 7 was assigned to those unable to discriminate at all. Exploratory analyses used separate ordinal regressions to confirm no difference in thresholds because of dependence severity, sex, or ethnicity. In secondary comparisons, subjective perceptions of the threshold and subthreshold cigarettes were calculated as the difference between effects of the higher nicotine content versus ultra-low cigarette during all trials in which they were administered during training and testing. The nonparametric Wilcoxon signed-rank test (*Z*) was used to analyze choice between the higher nicotine content and ultra-low nicotine cigarettes, within sessions. The absolute number of puff choices (out of 8 total) for each higher nicotine versus ultra-low nicotine cigarette was compared to gauge relative reinforcement from the threshold and subthreshold cigarettes differing in nicotine content. Similar comparisons (via *t*-tests) were

made of the difference in perceptions and choice for the 17 versus 0.4 mg/g in session 1 between those unable versus able to discriminate those cigarettes.

## Results

### Nicotine Discrimination Thresholds and Subthresholds

The distribution and frequencies of nicotine discrimination thresholds, compared to the 0.4 mg/g ultra-low, are shown in Figure 1. Demonstrating individual variability in sensitivity to nicotine discrimination, thresholds were found at each of the higher nicotine content Spectrum cigarettes versus 0.4 mg/g for 74% of participants (*n* = 45), from 1.3 mg/g up to 17 mg/g, with the remaining 26% (*n* = 16) unable to discriminate the highest content cigarette from 0.4 mg/g under these testing conditions. Focusing on discrimination between the lower nicotine cigarettes, fewer than 20% could discriminate 2.3 mg/g versus 0.4 mg/g. For the primary outcome result, the lowest threshold observed, four smokers, or about 7%, discriminated between the two lowest nicotine content Spectrum cigarettes of 1.3 mg/g versus 0.4 mg/g. Thus, the remaining participants, more than 90%, were unable to discriminate between the two lowest (1.3 mg/g and 0.4 mg/g ultra-low).

Exploratory analyses showed no association of FTND dependence score, OR = 0.84, 95% CI [0.65 to 1.09], Wald (1) = 1.70, *p* = .19, or age with odds of higher discrimination thresholds, OR = 0.01, 95% CI [−0.03 to 0.05], Wald (1) = 0.19, *p* = .66. We also found no differences in thresholds between women and men (reference group), OR = 1.21, 95% CI [0.49 to 2.98], Wald (1) = 0.18, ns, or because of ethnicity (ie, between African Americans and non-Hispanic whites [reference]), OR = 1.29, 95% CI [0.48 to 3.47], Wald (1) = 0.25.
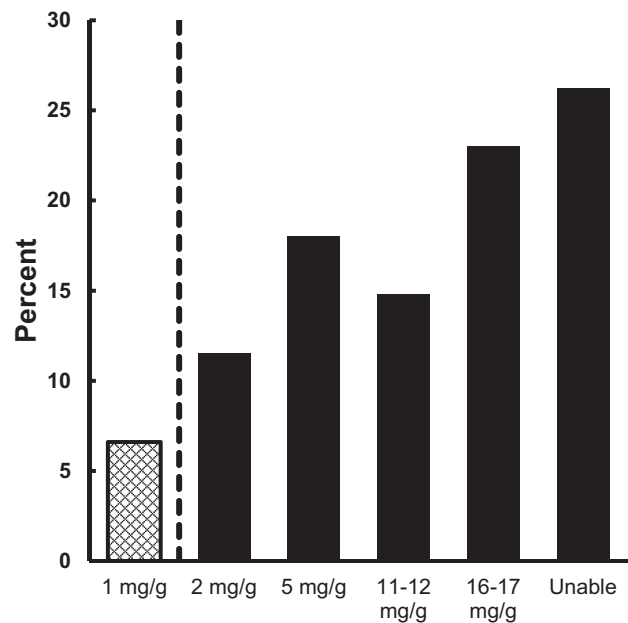


**Figure 1.** Percent of the 61 dependent smokers at each threshold nicotine discrimination content level (vs. 0.4 mg/g ultra-low), or who were unable to discriminate at all. Dotted line separates those (*n* = 4) with the lowest threshold, comprising less than 7% of the sample, from the higher thresholds found for the vast majority of smokers.
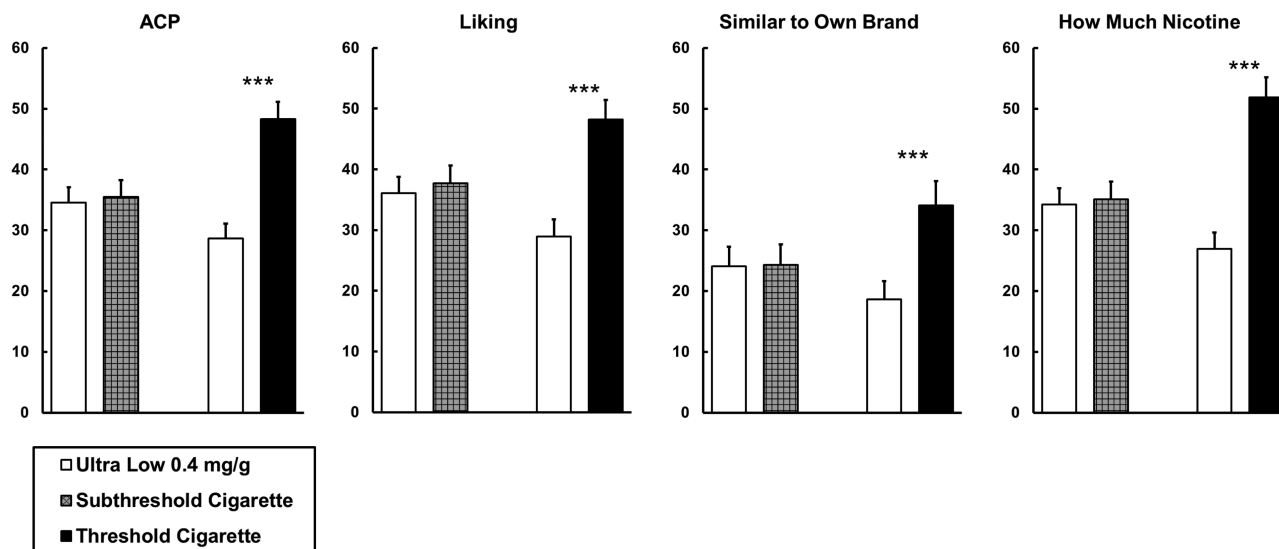
**Figure 2**. Mean (SEM) ratings on the Acute Cigarette Perception (ACP) scale of 5 items ("How much nicotine" and "Liking" shown here as example items), and on the separate "How similar to own brand" rating. Each was rated on a 0–100 visual analog scale in response to smoking their threshold or their subthreshold nicotine Spectrum cigarettes, with each tested in separate sessions versus the ultra-low (0.4 mg/g) comparison cigarette. Relative to the ultra-low, responses on each of these items were greater because of threshold (all *p*s < .001) but not because of subthreshold (all ns) cigarettes. Included here were those 42 smokers able to discriminate any of the higher nicotine content cigarettes from the 0.4 mg/g and not discriminate the next lowest, thus identifying their threshold and subthreshold cigarettes. (***\**p* < .001).

## Associations of Discrimination Thresholds With Perceptions and Choice Behavior

Subjective perceptions were related to discrimination threshold and subthreshold for the 42 smokers able to discriminate *and* to provide a subthreshold (ie, those who fail to discriminate the next lowest nicotine cigarette). Both ACP and "similar to own brand" ratings were significantly different between the threshold versus subthreshold cigarettes when each was compared to the 0.4 mg/g ultra-low, as shown in Figure 2. (Items of "how much nicotine" and "liking" are also shown for illustration of each individual ACP item). Notably, differences (increase) between the threshold cigarette versus 0.4 mg/g were significant for mean (SEM) ratings on the ACP composite scale (19.6 ± 3.4; paired *t* = 5.72, *p* < .001) and ratings for the "similar to own brand" item (18.5 ± 3.9, *t* = 4.70, *p* < .001), but those ratings were not different between the subthreshold versus 0.4 mg/g (0.9 ± 1.5 and 0.3 ± 1.4, respectively; both paired *t*s < 1). Results were the same for each of the 5 ACP items considered individually as well, as the differences in ratings between threshold and ultra-low cigarettes were significantly greater than the differences in ratings between the subthreshold and ultra-low (all paired *t*s > 3.58, all *p*s < .001).

Very similar results were found in the subsequent choice trials (Figure 3), as choice of the threshold nicotine cigarette was double that of the ultra-low (*Z* = 4.13, *p* < .001), but choice of the subthreshold cigarette was not different from that of the ultra-low (*Z* = 0.48, ns). Thus, as anticipated, cigarettes that could be discriminated from the 0.4 mg/g ultra-low were also subjectively perceived differently and were more reinforcing in the choice procedure, whereas cigarettes that could not be discriminated from the ultra-low were perceived similarly and were not more reinforcing.

Finally, and also consistent with the notion that discriminability is associated with perceptions and choice behavior, those 16 unable versus 45 able to discriminate the 17 mg/g versus 0.4 mg/g in session 1 reported significantly less increase in ACP score, means (SEM) of 9.3 ± 2.4 versus 24.7 ± 3.2, *t* = 2.74, *p* < .01, and then less increase in choice, 1.2 ± 0.5 versus 2.9 ± 0.4, *t* = 2.19, *p* < .05, for the 17 mg/g nicotine cigarette (difference from the 0.4 mg/g). However,
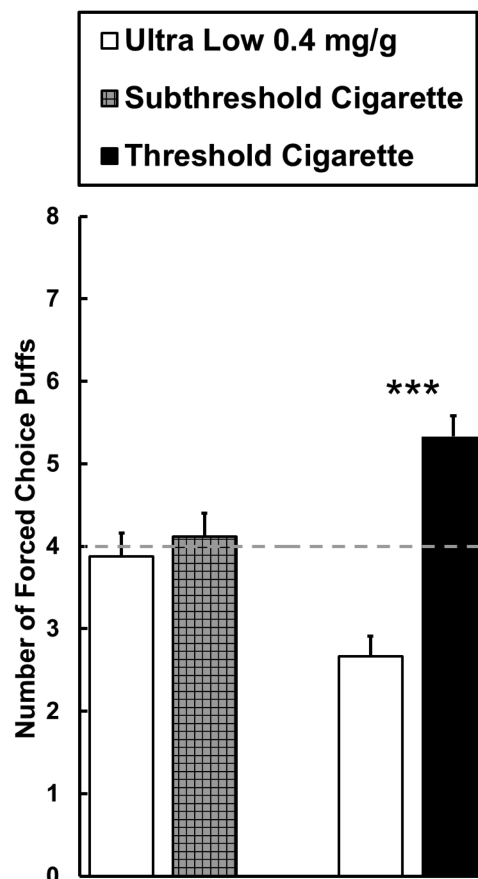


**Figure 3**. Choice of puffs (out of 8 total) for participants' discrimination threshold or subthreshold nicotine Spectrum cigarettes, each compared on separate sessions concurrently with the ultra-low (0.4 mg/g) cigarette in the subsequent choice trials (*N* = 42). (Dashed line at 4.0 signifies chance.) Relative to ultra-low, choice was greater for the threshold cigarette but not for subthreshold cigarette. (***\**p* < .001).

those unable versus able to discriminate did not differ on smoking characteristics of cigarettes per day, years smoking, age, or FTND. Very similar results were reported in our preliminary paper first describing this discrimination procedure with Spectrum cigarettes.[23]

## Discussion

Under the conditions of this research, dependent adult smokers vary widely in ability to discriminate higher nicotine cigarettes from the very lowest nicotine cigarette available. Most, 74%, were able to discriminate between cigarettes differing in nicotine content and identified a threshold nicotine content for discrimination from the 0.4 mg/g ultra-low. Most also identified a subthreshold (next lower) nicotine content cigarette. Greater subjective ratings and choice for the threshold cigarette are consistent with the notion that behavioral discrimination performance may be associated with acute self-reported perceptions of nicotine, which can then influence subsequent choice of the higher nicotine cigarette.[17] By contrast, similar ratings and choice of subthreshold versus ultra-low cigarettes suggests that inability to discriminate smaller differences in nicotine content reflects lack of perceptual differences between cigarettes, providing no motivation to choose one versus the other. Further supporting this notion was finding less increase in subjective ratings and choice for the 17 mg/g cigarette in session one among those unable versus able to discriminate the 17 mg/g versus 0.4 mg/g.

These findings lend credence to the rationale for establishing a maximum level of nicotine content in cigarettes that is below the discrimination threshold for the vast majority of smokers to aid reductions in tobacco dependence severity, thereby lessening the public health consequences of persistent smoking behavior. A cigarette that is not discriminated or reinforcing could, by definition, lead to inability of nicotine dependence to be initiated in teens or maintained in adults through cigarette smoking. Thus, any policy setting a maximum nicotine content cigarette at a level unlikely to maintain dependent smoking behavior could have to identify that level as one below that the vast majority of smokers would find reinforcing. In this sample of 61 smokers, only 7% were able, and about 93% were unable, to discriminate a cigarette with nicotine containing 1.3 mg/g over one with just 0.4 mg/g, the two lowest nicotine cigarettes available for comparison. On the basis of these discrimination results, then, the minimum *difference* in smoked nicotine content (1.3 vs. 0.4 mg/g) discriminable by these 7% was less than 1.0 mg/g. Lack of Spectrum cigarettes with nicotine contents intermediate between 1.3 and the 0.4 mg/g ultra-low precludes identifying a more precise threshold below 1.0 mg/g, which nearly all these smokers (>95%) would fail to discriminate (or perceive or choose).

The pattern of discrimination thresholds across this sample of dependent smokers needs to be replicated and confirmed as reliable with larger and more diverse samples to validate its generalizability across the population of smokers. Thresholds were found at every comparison cigarette tested versus the 0.4 mg/g for some of these smokers. This wide variability in nicotine discrimination thresholds is consistent with other clinical and preclinical research on individual differences in the dose-dependency of responses to nicotine,[36,37] including via Spectrum cigarettes.[38] Factors responsible for this variable sensitivity or responsivity to nicotine are unknown but warrant research attention; differences in FTND dependence, sex, and ethnicity were not related to nicotine discrimination responding in this study. On the other hand, our results may be specific to these

acute testing procedures which, during their development and evaluation, required increasing the number of training trials from 2 to 4 to enable more smokers to successfully discriminate cigarettes differing in nicotine contents.[23] Therefore, more exposures to the pairs of cigarettes compared here (ie, even more training trials) could lead many of the 26% of participants unable to discriminate in this study to subsequently learn to successfully discriminate the higher nicotine cigarettes from the 0.4 mg/g. Similarly, these procedural changes could lower nicotine thresholds in the other smokers, who were able to discriminate in this study.

Because of study limitations, far more research is needed to identify a level of nicotine content in cigarettes that the majority of smokers would not find reinforcing if it was the only one available,[6] which may better represent the regulatory environment in which they would find themselves if a low nicotine content standard were set for tobacco cigarettes. The current research always provided the 0.4 mg/g ultra-low, rather than no nicotine, as an alternative for testing discrimination from a higher nicotine cigarette, and controlled study of discrimination, perceptions, and self-administration of a cigarette below 1.0 mg/g nicotine that is the only one with nicotine available warrants research. Further development of other extremely low nicotine content cigarettes beyond just the 0.4 and 1.3 mg/g nicotine versions available from Spectrum would likely be important, along with a placebo cigarette for comparison in research studies (even if not allowed for commercial access because of current restrictions in federal law[1]). Clinical research suggests that exclusive use of Spectrum cigarettes with nicotine contents of 2.3 mg/g or less may reduce (but not eliminate) smoking behavior over weeks of access in dependent adults not attempting to quit or reduce their smoking.[6] Preclinical research has informed the procedures for clinical research on this question and may continue to do so.[11,39,40]

In conclusion, the vast majority of these dependent adult smokers were only able to discriminate cigarettes with far more than 1.3 mg/g nicotine, versus the 0.4 mg/g comparison, which were the two lowest content cigarettes available for study here. These results suggest a content at or below this difference of 0.9 mg/g may be indiscriminable by most smokers. Importantly, we observed that nicotine content cigarettes able to be discriminated (ie, threshold) from the 0.4 mg/g were also subjectively rated more pleasurably on the ACP and were reinforcing in the choice test, whereas cigarettes unable to be discriminated (subthreshold) were not rated differently or reinforcing, as hypothesized. Further research may identify the population subthreshold nicotine content in cigarettes below the level able to support persistent smoking behavior and dependence. Finally, procedures here to aid identification of thresholds for discriminating nicotine via tobacco smoking may be applicable to similar tests of discriminating nicotine administered by other rapid delivery methods, such as electronic cigarettes,[41] other combustible products, or other noncombustible products (eg, "heat not burn" cigarettes[42]).

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## Declaration of Interests

*None declared.*

## Acknowledgments

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