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This supplementary material has been provided by the authors to give readers additional information about their work.
1 eAPPENDIX 1. METHODS

1.1 Study Sample
Participants were 169 adult daily smokers (56 with affective disorders, 60 with opioid dependence, 53 socioeconomically disadvantaged women of reproductive age), who provided written informed consent. Participants were recruited through ads placed on Facebook, bulletin boards throughout the surrounding community, buses, as well as in local newspapers. Inclusion and exclusion criteria were chosen to align where possible with those used in the Donny et al. trial on reduced nicotine standards in cigarettes in the general population of smokers. Inclusion criteria that applied across subpopulations in the present study included being 18 years of age or older, reporting smoking five or more cigarettes per day, and providing an expired breath carbon monoxide (CO) level of more than 8 ppm (CoVita, Haddonfield, NJ). Participants also had to provide a negative urine toxicology screen for illicit drug use (i.e., amphetamine, methamphetamine, cocaine, barbiturates, benzodiazepines, buprenorphine, opiates, methadone, oxycodone, phencyclidine) except for marijuana (THC) (Rapid CHECK 9 panel Multi-Drug Test Card, and Single Panel Dipstrip for buprenorphine, Craig Medical, Vista, CA). Opioid-dependent participants were not expected to test negative for their prescribed medication (buprenorphine or methadone). All participants had to provide a breath alcohol level (BAL) at < .01 (Alco-Sensor IV, Intoximeter, Inc, St Louis, MO) at study intake assessment for inclusion in the study. Exclusion criteria that applied across subpopulations included intention to quit smoking within the next 30 days, use of other tobacco products on more than 9 of the previous 30 days, currently pregnant or trying to become pregnant, currently breastfeeding, exclusive use of “roll your own” cigarettes, and current suicidal ideation or recent suicide attempt (past year for those with affective disorders; past 10 years for women of reproductive age and opioid dependent).

Inclusion criteria specific to smokers with affective disorders were males and females ages 18-70 years, who met Mini International Neuropsychiatric Interview criteria for current or past year major depressive disorder, dysthymic disorder, generalized anxiety disorder, post-traumatic stress disorder, obsessive compulsive disorder, phobia or panic disorder with or without agoraphobia. Inclusion criteria specific to opioid-dependent smokers were males and females ages 18-70 years who were currently receiving methadone or buprenorphine maintenance treatment for opioid dependence. They had to be stable on their maintenance dose, which was defined as no change in dose in the past 30 days. They also had to have < 30% urine toxicology samples positive for illicit drug use in past month as confirmed by their provider. These stability criteria have been used in prior studies to protect against ongoing use of illicit drugs which can alter smoking rates and has been effective in prior studies in this population. Specific inclusion criteria for socioeconomically disadvantaged women of reproductive age were females only, ages 18-44 years with highest degree being high school or less. Educational attainment is an especially sensitive risk factor for smoking among women. We did not include younger women (15-17 yrs) to keep the study focus on adults as in the Donny et al trial while the upper age limit was to focus on women of reproductive age. Tobacco use among women of reproductive age is of considerable interest in tobacco control and regulatory science because of the serious adverse health risks to mother and child of use during pregnancy or during parenting of young children.

This study was approved by the local institutional review boards at each of the participating research sites (University of Vermont, Brown University, Johns Hopkins University) and was reviewed by the U.S. Food and Drug Administration (FDA) Center for Tobacco Products. Participants from each of the three populations were enrolled at the University of Vermont, with those with affective disorders enrolled at Brown University and those with opioid dependence and disadvantaged women enrolled at Johns Hopkins University School of Medicine.

1.2 Research Cigarettes
Study products were Spectrum research cigarettes manufactured by 22nd Century Group (Clarence, NY) and obtained from the National Institute on Drug Abuse following submission of
an application for an Investigational Tobacco Product with the Center for Tobacco Products, U.S. FDA. These products have been described previously. Four nicotine dose conditions were investigated using research cigarettes defined according to the nicotine content, averaged across menthol and non-menthol products (assignment of a menthol or non-menthol product was based on a participant’s reported usual brand): 15.8, 5.2, 2.4, and 0.4 mg of nicotine per gram of tobacco (mg/g). These cigarettes also differed in the content or yield of minor alkaloids and nitrosamines and in the application of casings, including sugars (which were higher in the 15.8 mg/g cigarettes than in the reduced-nicotine cigarettes in order to balance the ratio of nicotine to sugar). All sessions were conducted under double-blind conditions with the varying dose cigarettes being represented by letter codes. The dose and letter code combinations were determined randomly.

1.3 Procedure
Participants completed fourteen 2-4 hour experimental sessions (> 48 hours between sessions) in a within-subjects design. Experimental sessions were conducted in ventilated observation rooms (at least 4.3×5.9× 7.3 ft) equipped with Acer Aspire ES1-111 series laptops with 11.6” monitors that were used for completing questionnaires and for indicating cigarette preference in concurrent choices sessions (described below). Rooms were also equipped with Dell Optiplex 740 series computers that ran on Windows XP Professional and with 15” CRT monitors that were used for controlling smoke exposure (described below).

Experimental sessions were conducted following brief smoking abstinence (< 50% baseline breath CO level). Participants were instructed that they should try to abstain from smoking for at least 6-8 hrs in order to meet the breath CO criterion. Sessions were rescheduled if the abstinence criterion was not met. Experimental sessions could be scheduled in mornings, afternoons, or evenings, but were conducted at approximately the same time of day across sessions within individual participants. Upon arrival at the laboratory, participants completed a brief battery of physiological measures, including breath CO, BAL, urine toxicology screen for drugs of abuse, urine pregnancy test, weight, heart rate, and blood pressure. Experimental sessions were rescheduled for those who failed to meet the < 50% baseline breath CO criterion or had a BAL > .03%. Those with a positive drug screen were administered a field sobriety test: if passed the session was conducted and if failed the session was rescheduled. A positive pregnancy test resulted in discontinuation from the study.

At the beginning of each experimental session, participants were instructed to take two puffs from their usual brand cigarette under staff observation to equate time since last cigarette across study participants. Experimental sessions began 30 min following completion of the two puffs. During that 30-min wait period, participants completed the Minnesota Nicotine Withdrawal Scale (MNWS) and the Questionnaire of Smoking Urges-brief scale (QSU-brief). No eating or drinking other than water was permitted during sessions.

Briefly, the 14 sessions were organized into three phases: Phase 1 (Sessions 1-5) involved simulating demand for smoking each of the research cigarettes using the Cigarette Purchase Task (CPT) and assessments of subjective effects of smoking and smoking topography. Phase 2 (Sessions 6-11) involved assessment of preference between all dose-pairs of the four research cigarettes using free-operant concurrent choice procedures with each available at a relatively low response requirement (Fixed-Ratio 10). Phase 3 (Sessions 12-14) involved assessment of preference for lowest dose cigarette (0.4 mg/g) at the same low response requirement (Fixed -Ratio 10) vs. the 15.8 mg/g dose available on an 11-step progressive ratio schedule (10, 160, 320, 640, 1280, 2400, 3600, 4800, 6000, 7200, and 8400 where it was maintained until session completion).

1.3.1 Phase 1 (Sessions 1-5)
Participants smoked usual brand cigarettes in Session 1; in Sessions 2-5, participants were exposed to the different dose research cigarettes, one dose per session with order of exposure
randomized across sessions and participants. All cigarettes were smoked using a Clinical Research Support System (CReSS) Desktop smoking topography device (Borgwaldt KC, Richmond, VA). Individuals smoked each cigarette through a plastic cigarette holder that was attached to an air-filled tube, which leads to a pressure transducer. The device measures and records a number of smoking topography parameters, namely, (1) puff volume, (2) puff duration (3) maximum flow rate, and (4) total number of puffs.12 Each cigarette was smoked ad libitum to assess potential differences in smoking topography across the varying dose cigarettes.

Following completion of smoking the assigned cigarette in each session, participants were encouraged to make detailed notes about the cigarette (identified by letter code) which were available to them for use in Phases 2 and 3 when they had the opportunity to choose between smoking the different investigational cigarettes. Next, they completed the CPT9,10 and the modified Cigarette Evaluation Questionnaire (mCEQ)11. The CPT is a behavioral economic task measuring the relative reinforcing value of smoking in monetary terms (i.e., cigarette demand). Prior studies have shown that results are congruent across versions where participants consume purchased cigarettes as part of the experimental sessions and a hypothetical version where participants estimate how many cigarettes they would consume at varying prices.16 A hypothetical purchase task was used in the present study. Participants were instructed to imagine they had the same income/savings that they had right now, no access to any cigarettes or nicotine products other than those offered at these prices, they could smoke without any restrictions for the next 24 hours and they would smoke the cigarettes they requested at this time and could not save or stockpile cigarettes for a later date. Twenty prices per cigarette were assessed: $0.00 (free), $0.02, $0.05, $0.10, $0.20, $0.30, $0.40, $0.50, $0.60, $0.70, $0.80, $0.90, $1.00, $2.00, $3.00, $4.00, $5.00, $10.00, $20.00, and $40.00. At each price, participants were informed about the corresponding price per pack of cigarettes in addition to the price per cigarette. The mCEQ is a reliable and valid five-factor instrument that assesses subjective effects associated with the reinforcing and aversive effects of cigarette smoking.11

Unlike the CPT and mCEQ, which were completed once per session, participants provided expired CO samples and completed validated measures of nicotine withdrawal and craving (MNWS6, QSU-brief7), at 15-, 30-, 45-, and 60-min following completion of smoking.

1.3.2 Phase 2 (Sessions 6-11)
Upon arrival at the laboratory, participants completed the same battery of physiological measures as described above, took two puffs from their usual brand cigarette and completed the MNWS6 and QSU-brief7 questionnaires during the 30-min wait period between puffs and start of the experimental session. Upon initiation of the 3-hour experimental session, two different packs of research cigarettes were made available to participants, each with a different letter code (see eFigure 1). These letter codes corresponded to the same letter codes used with individual participants in the exposure sessions in Phase 1. Participants were encouraged to consult any notes they had made about each of the research cigarettes during the exposure sessions. Each of the possible 6 dose pairs was evaluated once per participant in random order and under double-blind conditions. Participants were instructed that they were free to choose to smoke either of the research cigarettes as often as they preferred and that they were also free to forego smoking either of the available cigarettes if that was their preference.11 The computer screen displayed two 1.25 inch squares with each having one of the two letter codes of the cigarettes available for that session embedded within each square. When participants wished to smoke, they used the computer mouse to direct the cursor to the desired square and associated letter code and clicked the mouse ten times (Fixed-Ratio 10). After completion of the response requirement, the screen changed colors displaying a printed instruction indicating that during the next three minutes the participant could take two puffs from the selected cigarette adhering to a controlled puffing protocol. Participants lit the cigarette without inhaling, inserted the cigarette into the cigarette holder filter, and then proceeded to begin puffing until a 60 mL volume of smoke had been inhaled which was displayed visually on the
computer screen by a counter that incremented as puff volume increased; a second counter immediately next to the running counter showed the goal volume of 60 mL. Participants were instructed to hold the inhaled puff in their lungs for 5 s that was also displayed on a running counter followed by a 25 s inter-puff interval, also displayed as a running counter on the computer screen, after which participants were to initiate a second puff following the same regimen. Once two puffs were taken from an earned cigarette, participants extinguished the cigarette and deposited the butt in a designated container for that cigarette code. They used a new cigarette for each subsequent two puffs earned. This controlled-puffing procedure is used so that differences in nicotine exposure that should be associated with smoking the varying dose cigarettes is not altered by between- or within-participant changes in smoking topography or confounding of dose and cigarette length at the time of smoking the different dose cigarettes. Upon completion of the session, participants completed the MNWS and the QSU-brief.

eFigure 1. Concurrent Choice Testing Arrangement

eFigure 1. During concurrent choice sessions in Phases 2 and 3 the left-most computer screen displayed two 1.25 inch squares with each having one of the two letter codes of the cigarettes available for that session embedded within each square (e.g., A & B as shown here). Participants could request to smoke by directing the cursor to the desired square and associated letter code and clicking the mouse the required number of times. After completion of the response requirement, the timer at the top of the left screen displayed a running counter showing the 3-min available for smoking. Participants took puffs off the cigarette through the plastic holder shown in center of figure, which recorded puff volume and duration. The upper row of counters on the right-most screen provided visual feedback on puff volume with the goal being 60 mL. The lower row of counters provided feedback on the goal of retaining the smoke in the lungs for 5 s followed by a 25 s inter-puff interval after which the participant could take a second puff following the same regimen.

1.3.3 Phase 3 (Sessions 12-14)
The experimental arrangement for these three sessions was the same as in Phase 2 except that the focus was on examining preference for the lowest dose cigarette (0.4 mg/g) available at a fixed relatively low response requirement (same 10 mouse clicks as in Phase 2) vs. the highest dose (15.8 mg/g) that was available on a progressive ratio schedule that began at the same 10-response requirement but incremented upwards each time it was chosen (10, 160, 320, 640, 1280, 2400, 3600, 4800, 6000, 7200, and 8400 clicks where it remained until session end). Choices to smoke resulted in the same opportunity to take 2 controlled puffs from the chosen cigarette as in Phase 2.
The same procedures were repeated across the three sessions comprising Phase 3 in order to refine the estimate of what response-requirement differential between the low and high doses would reverse preference for the latter dose and to discern possible changes over time in the development of that preference reversal. Following completion of each Phase 3 session, participants completed the CPT\textsuperscript{9,10} twice, once for each of the two dose options.

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**eFigure 2. Concurrent choice preference between two lowest doses across populations**

![Bar chart showing preference between two lowest doses across populations](image-url)
**eFigure 2.** Mean proportion of choices allocated to the 2.4 versus 0.4 mg/g nicotine dose cigarettes in each of the three study populations (affective disorder, disadvantaged women, opioid dependent). Data points are means across participants and error bars represent ± SEM. There was a significant difference between populations ($F_{2,154}=3.27, p=.04$) for this dose pair where choice of the higher dose was greater than chance among smokers with an affective disorder ($t_{154}=3.46, p<.001$), but not disadvantaged women ($t_{154}=1.92, p=.06$) nor those with opioid dependence ($t_{154}=0.11, p=.91$).

**eFigure 3.** Simulated demand across populations

**A**

Overall Demand for the 2.4 mg/g dose at escalating prices across the three populations of interest (opioid dependent, affective disorders, disadvantaged women). Data points are means across participants, with shaded areas representing the 95% CI in the best lines. Overall Demand among those with opioid dependence was significantly more inelastic (greater persistence in demand) than among disadvantaged women ($F_{1,38}=21.00, p<.001$) or those with affective disorders ($F_{1,38}=20.00, p<.001$). Panel B: Demand Intensity (estimated consumption at $0 price). Data points are means across participants for each of the three populations with error bars representing ± SEM; data points not sharing a letter code differ significantly after Bonferroni correction. Demand Intensity differed by population ($F_{2,97}=5.02, p=.008$), with greater demand among those with opioid dependence compared to disadvantaged women ($t_{154}=3.02, p=.009$) although not

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those with affective disorders ($t_{92}=2.22$, $p=.09$). There was no difference between disadvantaged women and those with affective disorders ($t_{92}=0.37$, $p=.99$).

eFigure 4. Simulated demand for highest and lowest nicotine content cigarettes

eFigure 4. Simulated demand for the 0.4 mg/g and 15.8 mg/g dose cigarettes in the Cigarette Purchase Task that was completed during each of the Phase 3 sessions (Sessions 12-14). Data points are means across participants, with shaded areas representing the 95% CIs for the best-fit lines. An exponential demand equation$^{18}$ described demand well across both doses with $R^2=.97$. Demand for the 15.8 mg/g dose was
significantly greater than for the 0.4 mg/g dose ($F_{1,38}=7.45$, $p=.01$). There were no significant differences across sessions or populations in these relationships.

eTable 1. Time-course of effects of the varying dose research cigarettes on mean (SEM) Questionnaire of Smoking Urges-brief (QSU-brief) Factor 1 and 2 scores

<table>
<thead>
<tr>
<th>Research Cigarettes</th>
<th>0.4 mg/g</th>
<th>2.4 mg/g</th>
<th>5.2 mg/g</th>
<th>15.8 mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Smoking Baseline</td>
<td>5.9 (0.1)$^{a1}$</td>
<td>5.9 (0.1)$^{a1}$</td>
<td>5.9 (0.1)$^{a1}$</td>
<td>6.0 (0.1)$^{a1}$</td>
</tr>
<tr>
<td>+15 Minutes</td>
<td>4.7 (0.1)$^{a2}$</td>
<td>4.5 (0.1)$^{ab2}$</td>
<td>4.3 (0.1)$^{b2}$</td>
<td>3.9 (0.1)$^{b2}$</td>
</tr>
<tr>
<td>+30 Minutes</td>
<td>5.0 (0.1)$^{a3}$</td>
<td>4.9 (0.1)$^{a3}$</td>
<td>4.7 (0.1)$^{a3}$</td>
<td>4.3 (0.1)$^{a3}$</td>
</tr>
<tr>
<td>+45 Minutes</td>
<td>5.2 (0.1)$^{a3,4}$</td>
<td>5.2 (0.1)$^{a3,4}$</td>
<td>5.2 (0.1)$^{a4}$</td>
<td>4.8 (0.1)$^{b4}$</td>
</tr>
<tr>
<td>+60 Minutes</td>
<td>5.6 (0.1)$^{a4}$</td>
<td>5.5 (0.1)$^{a4}$</td>
<td>5.5 (0.1)$^{a4}$</td>
<td>5.2 (0.1)$^{a5}$</td>
</tr>
<tr>
<td><strong>Factor 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Smoking Baseline</td>
<td>3.7 (0.1)$^{a1}$</td>
<td>3.7 (0.1)$^{a1}$</td>
<td>3.7 (0.1)$^{a1}$</td>
<td>3.9 (0.1)$^{a1}$</td>
</tr>
<tr>
<td>+15 Minutes</td>
<td>2.8 (0.1)$^{a2}$</td>
<td>2.8 (0.1)$^{a2}$</td>
<td>2.7 (0.1)$^{ab2}$</td>
<td>2.4 (0.1)$^{b2}$</td>
</tr>
<tr>
<td>+30 Minutes</td>
<td>3.0 (0.1)$^{a2}$</td>
<td>3.0 (0.1)$^{a2}$</td>
<td>2.9 (0.1)$^{ab2}$</td>
<td>2.7 (0.1)$^{b2}$</td>
</tr>
<tr>
<td>+45 Minutes</td>
<td>3.3 (0.1)$^{a3,4}$</td>
<td>3.2 (0.1)$^{a3,4}$</td>
<td>3.2 (0.1)$^{a3}$</td>
<td>3.0 (0.1)$^{a4}$</td>
</tr>
<tr>
<td>+60 Minutes</td>
<td>3.4 (0.1)$^{a3}$</td>
<td>3.4 (0.1)$^{a4}$</td>
<td>3.4 (0.1)$^{a3}$</td>
<td>3.2 (0.1)$^{a4}$</td>
</tr>
</tbody>
</table>

Note. Mean Questionnaire of Smoking Urges-brief (QSU-brief) Factor 1 and Factor 2 scores across the four nicotine doses and time-course of assessments. The dose-by-time interaction was significant for both QSU factors ($F_{12,2014}=8.92$, $p<0.001$ and $F_{12,2014}=5.22$, $p<0.001$ for Factor 1 and Factor 2, respectively). Effects of time and dose for QSU Factor 1 were $F_{4,672}=144.86$, $p<0.001$ and $F_{3,501}=11.22$, $p<0.001$, respectively, while the effects of time and dose for Factor 2 were $F_{4,672}=118.77$, $p<0.001$ and $F_{3,501}=4.09$, $p=0.007$, respectively. There were no differences across populations in either QSU Factor 1 ($F_{2,166}=0.59$, $p=0.56$) or QSU Factor 2 ($F_{2,94}=0.95$, $p=0.39$) ratings. Within each time point, data points not sharing a letter differ significantly after Bonferroni correction. Within each dose, data points not sharing a number differ significantly after Bonferroni correction.

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Mean Puff Volume

Mean Puff Duration

Mean Maximum Flow

Puff Number

eFigure 5. Mean (± SEM) puff volume, puff duration, flow rate, and puff number observed during ad-libitum smoking of research cigarettes with varying nicotine content levels (0.4, 2.4, 5.2, and 15.8 mg/g tobacco); data points not sharing a letter differ significantly after Bonferroni correction. There was no significant effect of dose, population, nor interactions of dose and population on puff volume or puff duration (F_{3,486}=0.76, p=0.52; F_{2.85}=2.77, p=0.07; F_{6.49}=0.66, p = 0.68 for effects of dose, population, and dose-by-population interaction, respectively, for puff volume and F_{3.486}=0.54, p=0.65; F_{2.109}=1.88, p=0.16; F_{6.481}=0.88, p = 0.51 for effects of dose, population, and dose-by-population interaction, respectively, for puff duration). There was a significant main effect of dose on maximum flow rate (F_{3,488}=3.55, p = 0.02), but no significant population differences (F_{2.106}=1.02, p=0.36), nor interactions of dose and population (F_{6.49}=0.47, p=0.83). There was also a main effect of dose on puff number (F_{3,487}=17.96, p<0.001), but no significant effect of population (F_{2.108}=0.04, p=0.96) nor interaction of dose and population (F_{6.481}=0.76, p=0.61). Data points not sharing a letter differ significantly after Bonferroni correction.

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eTable 2. Time-course of effects of the varying dose research cigarettes on mean (SEM) breath carbon monoxide boost following acute exposure

<table>
<thead>
<tr>
<th></th>
<th>Research Cigarettes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4mg/g</td>
</tr>
<tr>
<td>+15 minutes*</td>
<td>5.7 (0.25)</td>
</tr>
<tr>
<td>+30 minutes*</td>
<td>5.1 (0.23)</td>
</tr>
<tr>
<td>+45 minutes*</td>
<td>4.8 (0.22)</td>
</tr>
<tr>
<td>+60 minutes*</td>
<td>4.4 (0.21)</td>
</tr>
</tbody>
</table>

Note. Asterisk (*) denotes significant main effects of time (F_{3,504}=104.46, p<0.001). There were no significant main effects of dose, population, nor interactions of time, dose, or population (F_{3,399}=0.92, p=0.431, F_{2,166}=0.10, p = 0.91, and F_{18,1494}=1.07, p=0.38, respectively).
REFERENCES


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