

Smoking in the absence of nicotine: behavioral, subjective and physiological effects over 11 days

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ABSTRACT

Aims Sensorimotor stimuli associated with tobacco smoking influence smoking behavior; however, current research has focused almost exclusively on the effects of brief, laboratory-based exposure to smoking-related stimuli. The purpose of this experiment was to characterize the effects of smoking stimuli delivered in the absence of nicotine over an extended (11-day) exposure. **Design, setting and participants** Thirty adult regular smokers participated in an in-patient study. After assessing preferred brand smoking, participants were assigned randomly to one of three groups corresponding to subsequent smoking conditions: nicotine-containing cigarettes, de-nicotinized cigarettes or no smoking. **Measurements** Measures of smoking reinforcement, subjective effects, physiological effects, withdrawal/craving and puff topography were taken repeatedly during both periods of free access and controlled assessments during abstinence. **Findings** Daily de-nicotinized cigarette use declined immediately by 1.7 cigarettes/day compared to the preferred brand baseline and declined by another 3.5 cigarettes over time; participants smoking de-nicotinized cigarettes also demonstrated a 31% decline in the number of puffs earned on a progressive ratio, a measure of the motivation to smoke, during the study. Subjective ratings of smoking were largely negative throughout the study in the de-nicotinized group, while the nicotine-containing condition reported increasingly positive subjective effects with repeated exposure. Acute craving suppression following smoking remained evident throughout the study regardless of nicotine content. **Conclusions** These effects highlight the importance of non-nicotine sensorimotor stimuli as determinants of the maintenance of smoking behavior and suggests that extinction of conditioned reinforcement in the absence of nicotine progresses slowly.

Keywords Cigarettes, conditioning, de-nicotinized, extinction, nicotine, reinforcement, reward.

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INTRODUCTION

It is generally accepted that nicotine is a necessary constituent in tobacco driving smoking [1]. However, converging molecular, neuropharmacological, behavioral and clinical evidence suggests that the primary reinforcing consequences of nicotine are not a sufficient explanation for smoking [2–4]. Much of this research has focused on sensorimotor smoking stimuli as critical determinants of smoking.

The sensory stimuli produced by cigarette smoke are distinct, salient and consistently paired with nicotine, providing an ideal context for establishing a conditioned association with nicotine [5]. Presumably as a consequence of this association, smoking stimuli acquire

properties that contribute to smoking. Indeed, de-nicotinized and nicotine-containing cigarettes result in similar levels of smoking during brief laboratory sessions [6], similar decreases in subsequent preferred brand smoking [7], similar responding on a progressive ratio schedule of reinforcement [8,9] and similar decreases in withdrawal and craving [6]. It is also clear that nicotine adds to smoking effects; nicotine-containing cigarettes are preferred over de-nicotinized cigarettes when they are concurrently available [10], produce greater positive subjective effects [7,11–13] and alleviate a wider range of withdrawal symptoms [14].

Together, this research indicates that sensorimotor smoking stimuli may be important for understanding smoking behavior and consequences. However, most

human research to date has focused on the acute effects of smoking stimuli and their role in short bouts of smoking. Little is known about whether or to what extent these effects are maintained over prolonged exposure. If sensorimotor smoking stimuli acquire their properties through Pavlovian conditioning, their continued presentation in the absence of nicotine should lead to a loss of their effects (i.e. extinction). Recently, Rose & Behm [15] found that exposure to de-nicotinized cigarettes for 2 weeks reduced the subjective reward associated with usual-brand smoking, suggesting that the positive subjective effects of smoking stimuli can be extinguished with repeated exposure and that this process generalizes to preferred brand cigarettes. In contrast, Buchhalter and colleagues [14] found little evidence that the ability of de-nicotinized cigarettes to suppress withdrawal and craving dissipated over 5 days. While smoking behavior was not measured directly, participants turned in fewer cigarette butts during the de-nicotinized condition and had lower expired carbon monoxide (CO) levels than when they had access to nicotine-containing cigarettes [14]. These differences were stable over time, suggesting reduced reinforcement in the de-nicotinized condition, but little evidence of further extinction over the 5-day period.

The purpose of the present study was to examine further whether the effects of de-nicotinized cigarettes dissipate with repeated exposure. We utilized an in-patient design to maximize the extinction process by maintaining a stable and novel context in which new learning could occur [16] and ensure compliance with the smoking conditions [14,15]. A broad range of constructs (e.g. reinforcement, motivation to smoke, subjective effects, mood, withdrawal/craving and puff topography) were assessed repeatedly, allowing us to characterize change thoroughly over time.

METHODS

Participants

Thirty adult, out-patient, volunteers were recruited through advertising, consented to participate and completed the study. Five additional participants did not complete the study. Three of the five left prior to randomization; two left after being randomized to abstinence. The inclusion criteria were as follows: 18–65 years of age, self-reported smoking of 10 or more cigarettes per day for the last year, inhaling while smoking, no intention to quit in the next 3 months and a drug-free urine at screening (opiates, cocaine and benzodiazepines). Exclusion criteria were as follows: significant medical illness, evidence of a major psychiatric illness within past 6 months, females who are pregnant or

lactating, current drug abuse treatment, drug dependence (excluding nicotine and caffeine) and diagnosed sleep disorder. The sample was 53% female and predominantly African American (72%). Most participants (93%) completed high school or obtained a general equivalency diploma. Participants, on average, were 37.8 years of age (SD: 11.4), smoked 21.4 (SD: 11.8) cigarettes per day and scored 5.3 (SD: 1.9) on the Fagerström Test for Nicotine Dependence (FTND) (17). Most (77%) reported that their preferred brand was mentholated.

Design

This study employed a between-subjects, double-blind design. We utilized a between-subjects design to limit unblinding resulting from direct comparison of the two experimental cigarettes. Volunteers first participated in a telephone and an in-person screening. If interested and they qualified, participants were admitted to a residential research unit at the Johns Hopkins Bayview Medical Center for 13 nights. Participants were paid for their time and inconvenience.

Procedures

For the first 2 days, all volunteers were allowed to smoke their preferred brand of cigarettes without restriction. On day 1, participants were familiarized with the subjective and behavioral measures. Day 2 served as a baseline assessment of preferred brand smoking; the assessments conducted on day 2 were identical to those taken on days 4–7 and 9–12. On these days (i.e. days 2, 4–7, 9–12), volunteers were allowed to smoke without restriction between 6 a.m. and 10 p.m.; smoking was not allowed between 10 p.m. and 6 a.m. On day 3, 10 participants were assigned randomly to each of three smoking conditions [no smoking, de-nicotinized cigarettes (Denic) and nicotine-containing cigarettes (Nic)] and remained in those conditions throughout the rest of the study. Days 3, 8 and 13 tested the effects of smoking the assigned cigarette (day 3) and change in these effects (days 8 and 13) under controlled conditions. On these days, volunteers were allowed to smoke during only two laboratory sessions (controlled puffing, progressive ratio); unrestricted smoking did not resume until the following day.

Unrestricted smoking assessments

All participants were allowed to smoke their preferred brand of cigarette freely on day 2, and participants in the Denic and Nic conditions were allowed to smoke their assigned cigarettes freely on days 4–7 and 9–12. The first three cigarettes of each day were dispensed from the nurse's station to aid in the collection of subjective and topography data. The subjective effects of smoking,

craving, CO and cardiovascular measures were assessed before and/or after the first and third cigarette. On days 2, 4, 6, 9 and 11, participants also smoked their first and third cigarettes through the portable topography device (CReSSmicro, Plowshare Technologies, Baltimore, MD, USA). Following the third cigarette, volunteers were given an ample supply of cigarettes for the remainder of the day. Volunteers were responsible for tracking their own cigarette consumption, including recording the time each cigarette was smoked and retaining the unsmoked portion of each cigarette. Cigarette logs were checked against the number of cigarette butts collected daily.

Controlled puffing procedure

On days 3, 8 and 13 (9.00–10.00 a.m.), participants in the Denic and Nic groups inhaled eight 30 cc puffs at an interpuff interval of 1 minute, holding each puff for 3 seconds. Puffing was controlled using real time auditory and visual feedback from desktop topography equipment (CReSS, Plowshare Technologies). Actual volume closely approximated the target volume of 30 cc and did not differ between smoking conditions. Subjective effects, CO and cardiovascular measures were assessed before and/or after smoking. Participants in the no smoking condition read quietly during the time reserved for smoking in the other two groups. Heart rate and blood pressure were taken throughout the session. Participants were instructed that the same cigarettes, inhalation volume and inhalation duration would be available for them later that afternoon in the progressive ratio session.

Progressive ratio procedure

On days 3, 8 and 13 (2.00–5.00 p.m.), participants could earn 30 cc puffs from their assigned cigarettes by pressing the button on the mouse. The number of responses required for each puff increased with each puff earned as follows: 50, 250, 500, 1000, 1500, 2000, 2500, 3000, 3500 and 4000. A minimum inter-response interval of 0.5 seconds was enforced; responding at a faster rate did not count towards completion of the schedule requirements. Volunteers had 3 hours to earn as many puffs as they desired. Puffs were taken immediately after completing each response requirement. Responding was allowed to resume 1 minute after completing the preceding response requirement. Participants in the no smoking condition were allowed to respond, but there was no consequence for completing the ratio requirement. All participants could participate in a neutral activity (e.g. reading) during this time to minimize the influence of boredom on responding.

Additional measures

An extended battery of withdrawal, craving, mood and sleep assessments was administered at 2 p.m. on days 2,

4–7 and 9–12. On day 13, participants were administered an end-of-study questionnaire.

Study cigarettes

Quest brand cigarettes (Vector Tobacco Inc., Research Park Triangle, NC, USA) were used as the study cigarettes. Cigarettes were freely available and all brand labeling was hidden from the staff and volunteers. Nicotine-containing cigarettes (Quest 1) contained 0.6 mg of nicotine and 10 mg of tar and de-nicotinized cigarettes (Quest 3) contained 0.05 mg of nicotine and 10 mg of tar, as measured by the Federal Trade Commission method. It is important to note that nicotine delivery was not directly measured. Menthol versions of both types of cigarettes became available after the study was initiated. Three menthol-preferring participants in both the Nic and Denic groups were assigned to non-mentholated study cigarettes prior to the availability of Quest menthols. Menthol smokers were not excluded because of high rates of menthol smoking in the Baltimore area. Inspection of the data revealed that the pattern of smoking observed was similar in menthol-matched and menthol-mismatched participants. Given the small number of participants, the similarity in observed effects on smoking and the fact that the number of participants who were menthol mismatched was equal in the smoking groups, all individuals were included in the analyses.

Measures

Smoking behavior

Data collected on the time of each cigarette smoked was compiled into three distinct variables: the total number of cigarettes smoked per day, the latency to the first cigarette of the day (i.e. from time of awakening) and the pattern of daily smoking (i.e. cigarettes smoked per 4-hour period of availability). In addition, the average weight of the unsmoked cigarettes was calculated for each day.

Puff topography

Characteristics of puffing behavior were assessed by determining the puff volume, puff duration, peak flow rate, average flow rate, interpuff interval and number of puffs per cigarette. Although volume, duration and flow are measured with each puff, the data reported below represent the mean across puffs for each cigarette.

Cigarettes effects questionnaire

Visual analog scales (0–100; anchored by 'not at all' and 'extremely') assessed the effects of smoking on: satisfying, pleasant, unpleasant, like taste, dislike taste, smoke versus air (anchored with 'mostly smoke' to 'mostly air'), harsh, strength, high in nicotine, like drug effect, dislike

drug effect, like cigarette, dislike cigarette, calming, relaxing, comforting, less irritable, sense of wellbeing, more awake, easier to concentrate, exhilarating, pleasurable excitement, dizziness, lightheaded, nauseating and nervous [18].

Withdrawal and craving questionnaires

Withdrawal and craving were assessed using standard, validated scales. The Shiffman–Jarvik Withdrawal Scale (SJWS) [19] assesses symptoms of nicotine withdrawal with five subscales: craving, psychological discomfort, physical discomfort, stimulation/sedation and appetite. The Questionnaire on Smoking Urges (QSU) [20] assessing craving using two factors (factor 1: intention to smoke; factor 2: anticipation of relief from withdrawal). The Schuh–Stitzer VAS Craving Scale [21], a five-item visual analog scale, was used as an alternative to the QSU to assess changes in craving after the first and third cigarette on unrestricted smoking days because of its brevity and the desire to reduce task demands.

Mood questionnaires

The Profile of Mood States (POMS) [22] and the Positive and Negative Affect Scale (PANAS) [23] were completed on days 2, 4–7 and 9–12 during the afternoon battery to assess mood.

Sleep questionnaire

The St Mary's Sleep Questionnaire [24] was administered daily and includes measures related to the duration of sleep, time to fall asleep, number of awakenings and subjective assessments of depth of sleep, sleep quality, sleep satisfaction, clear-headedness after awakening, difficulty getting to sleep and difficulty staying asleep.

Physiological measures

Heart rate and blood pressure were measured (non-invasive patient monitor model 506; Criticare Systems, Waukesha, WI, USA) throughout the controlled puffing (9–10 a.m.) session. Expired air CO was taken at 10 a.m., 4 p.m. and 10 p.m. on days 1, 2, 4–7 and 9–12. CO was also assessed hourly from 8 a.m. to 10 p.m. on days 3, 8 and 13 to ensure compliance with smoking restrictions. All CO assessments were taken a minimum of 2 minutes after the last cigarette. Saliva samples were collected each evening at 10 p.m. These samples were not analyzed, but instead used as a bogus pipeline to further deter smoking of non-study cigarettes.

End of study questionnaire

As part of their study evaluation, participants in the Denic and Nic groups were asked the following question:

'In your estimation how much nicotine was in the cigarettes you were asked to smoke?' on a five-point scale. Response categories were (1) nicotine-free: 0 mg; (2) ultra lights: 0.4 mg (e.g. True, Merit ultra lights); (3) lights: 0.8 mg (e.g. Marlboro Light, Vantage); (4) full flavor: 1.1 mg (e.g. Marlboro, Camel); and (4) very strong: 1.7 mg (e.g. Pall Mall).

Data analysis

Data were analyzed for baseline group differences (day 2 only). Data from days 3–13 were analyzed both as raw scores (not including baseline) and after adjusting for baseline (i.e. difference from baseline scores). The results of the difference score analyses are presented only if there were significant group differences at baseline or if the effects of group differed in important ways from the raw data. All data were analyzed using Proc Mixed (SAS) with one or more of the following factors: group, day, cigarette, time of day and pre/post smoking. The repeated-measures variable day was incorporated into the model as a linear and quadratic polynomial contrast for measures taken during unrestricted smoking (i.e. when the number of assessments was large enough to estimate the nature of the change over time). Additional analyses of individual smoking groups were conducted to directly compare change in these groups and to further describe change within each group. Planned comparisons of the different groups within a given day and time were made using *t*-tests. Illustrative examples of effect size are reported as Cohen's *d*. Data reported below focus on main and interaction group effects; other effects are not reported for brevity. Differences with a probability of $P < 0.05$ were considered statistically significant.

RESULTS

Unrestricted smoking behavior

Prior to randomization (i.e. day 2), participants in the three conditions smoked their preferred cigarettes similarly (Fig. 1). Although participants subsequently assigned to the no smoking condition tended to smoke more cigarettes at baseline than did other groups, this trend was driven by a single individual who smoked 37 cigarettes on day 2. Analysis of puff topography taken after the first and third cigarettes of the day revealed a main effect of subsequent group assignment on puff volume ($P = 0.011$) and puff duration ($P = 0.049$). Participants subsequently assigned to the no smoking condition had lower average puff volume ($P_s < 0.05$) and puff duration ($P_s < 0.05$) than each of the other groups. Importantly, there were no significant differences between the two subsequent smoking conditions at baseline.

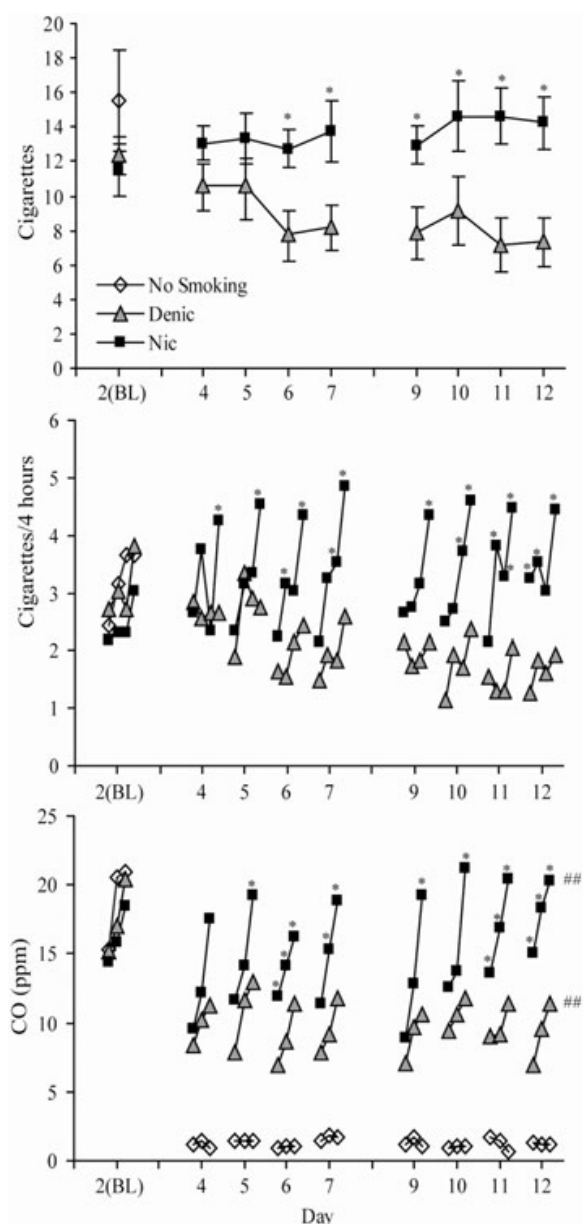


Figure 1 Upper panel: mean (\pm SEM) total number of cigarettes smoked during each unrestricted smoking day. Middle panel: mean number of cigarettes smoked per 4-hour period for each unrestricted smoking day. Adjacent points within each day represent 6 a.m.–10 a.m., 10 a.m.–2 p.m., 2 p.m.–6 p.m. and 6 p.m.–10 p.m., respectively. Lower panel: mean expired CO at three times each day; adjacent points represent 10 a.m., 4 p.m. and 10 p.m., respectively. In all panels, day 2 represents baseline, preferred brand smoking. Participants were randomized on day 3. SEMs are not shown for the middle and lower panel to increase clarity of the figure. *Significant difference compared to Denic. ##Significant differences at all post-baseline time-points compared to no smoking

After randomization, participants in the Nic group smoked more cigarettes and this difference grew over days (Fig. 1). Analysis of the number of cigarettes smoked per day revealed a main effect of group ($P = 0.013$) that was more robust after controlling for

baseline ($P < 0.001$). Participants assigned to the Nic group tended to increase the number of cigarettes smoked per day relative to their preferred brand by 2.1 cigarettes (i.e. collapsed across days; $d = 0.46$; $P = 0.13$), while participants in the Denic condition significantly decreased their cigarette consumption by 3.8 cigarettes ($d = 0.80$; $P = 0.041$). A linear group \times day interaction ($F_{1,126} = 9.19$, $P < 0.005$) was seen with a significant linear decrease in smoking over days in the Denic group ($P < 0.005$), but no significant change over time in the Nic condition. There were no main or interaction effects of group on puff volume, puff duration, interpuff interval, peak flow or average flow. Puff volume increased relative to baseline, preferred brand smoking, when participants were assigned to either the Denic (+4.8 cc; $d = 0.39$; $P = 0.024$) or Nic condition (+6.8 cc; $d = 0.25$; $P = 0.038$). However, individuals smoking nicotine-containing cigarettes took more puffs per cigarette (11.5 versus 8.8; $d = 0.65$; $P = 0.010$) and tended to leave less of their cigarettes unsmoked (0.45 g versus 0.54 g; $d = 0.47$; $P = 0.11$) than individual smoking de-nicotized cigarettes. Differences in cigarette butt weight reached significance after controlling for baseline.

Latency to smoke the first cigarette of the day tended to be longer for de-nicotized than nicotine-containing cigarettes. The change in latency to smoke was abrupt (i.e. seen on day 4) and did not change significantly over repeated days in either cigarette condition. Latencies increased by a mean of 55.8 minutes compared to baseline in the Denic condition while decreasing by a mean of 25.5 minutes in the Nic condition. This difference between groups in the latency to smoke failed to reach significance in the analysis of the raw data ($P = 0.09$), but did reach significance after adjusting for baseline ($d = 0.76$; $P = 0.013$).

The two smoking conditions also differed in the pattern of smoking within the day (Fig. 1). Analysis showed a significant effect of group ($P = 0.008$), a linear day \times group interaction ($P = 0.001$) and a linear time of day \times group interaction ($P < 0.001$). The within-day increase in smoking rate was much more pronounced for the Nic than for the Denic group; these group differences in within-day smoking patterns did not change over days of exposure.

Biochemical analysis verified the self-report smoking data. CO differed across groups ($P < 0.001$) with both linear day by group ($P = 0.035$) and linear time of day \times group interactions ($P < 0.001$). Contrasts focused on the two smoking groups indicated that the Nic group had higher CO levels than the Denic group overall ($P = 0.026$) and that the differences between the smoking groups increased during the day ($P < 0.001$; Fig. 1).

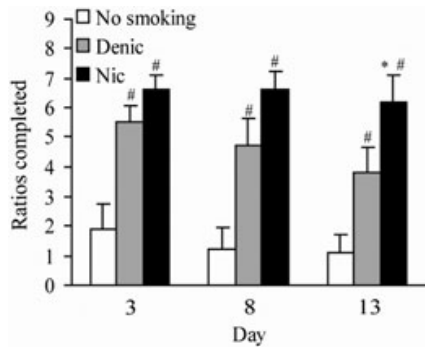


Figure 2 Mean (\pm SEM) total number of puffs earned during the progressive ratio procedure on days 3, 8 and 13. *Significant difference compared to Denic. #Significant differences compared to no smoking

Progressive ratio

The number of ratios completed during the progressive ratio procedure (Fig. 2) differed by group ($P < 0.001$). Both smoking conditions completed significantly more ratios across days than the no smoking condition ($P_s < 0.005$) and there was a trend for the two smoking conditions to differ from each other ($P = 0.058$). Pairwise comparisons revealed differences between each of the smoking conditions and no smoking at each day and a significant decline (i.e. day 3 versus day 13) in the Denic group ($d = 0.73$; $P = 0.025$) that was not observed in either of the other groups. The latter observation contributed to a significant difference between the Nic and Denic condition on day 13 ($d = 0.87$; $P = 0.027$).

Cigarette characteristic ratings

There were no main effects of group on the cigarette effects questionnaire at baseline. There was a significant group \times cigarette type interaction for ratings of the item dislike drug effect ($P = 0.004$). Pairwise comparisons revealed that ratings of dislike drug effect were higher in the Nic than both the no smoking and Denic groups after the third cigarette ($P_s < 0.05$).

After randomization, analysis of the raw data from cigarettes 1 and 3 on unrestricted smoking days indicated that participants assigned to the Nic group reported significantly higher ratings of pleasant ($P = 0.004$), satisfying ($P = 0.009$), like cigarette ($P = 0.012$) and enjoyable (Fig. 3; $P = 0.007$), and lower ratings of unpleasant (Fig. 3; $P = 0.012$), harsh ($P = 0.034$) and dislike cigarette ($P = 0.007$). Furthermore, some differences emerged or increased over days. These included significant group differences in the linear effect of day on pleasant ($P = 0.043$), calming ($P = 0.043$), less irritable ($P = 0.048$), more awake ($P = 0.033$) and enjoyable ($P = 0.030$) and trends for dislike drug effect, satisfying,

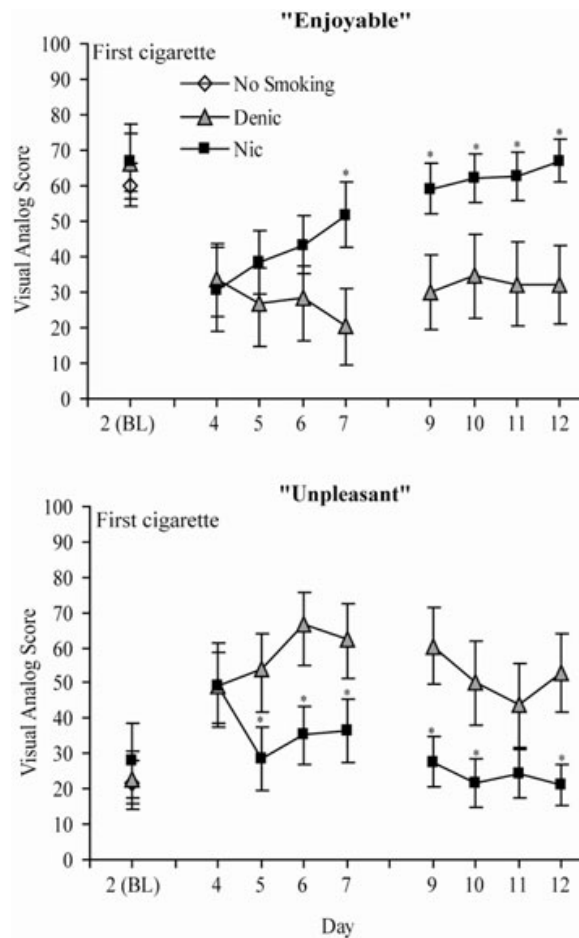


Figure 3 Mean (\pm SEM) visual analog ratings of how 'enjoyable' (upper panel) and 'unpleasant' (lower panel) smoking the first cigarette was on each of the unrestricted smoking days. Day 2 represents baseline, preferred brand smoking. Participants were randomized on day 3. *Significant difference compared to Denic

like cigarette and comforting ($P_s < 0.10$). There was also a significant quadratic day \times group interaction on like cigarette ($P = 0.034$) and dislike drug effect ($P = 0.033$) and trends for dislike cigarette and enjoyable ($P_s < 0.10$). For all group \times day interactions except dislike drug effect and dislike cigarette, ratings were higher in the Nic group and these differences increased over days. Analysis of the linear and quadratic effects of day within the Denic condition generally confirmed that there was little change over days; there was a linear decrease in lightheadedness ($P < 0.05$) and an increase in dislike drug effect that slowed over time (quadratic effect; $P < 0.05$). In contrast, analysis of the Nic group revealed significant linear increases in pleasant, like taste, satisfying, calming, like cigarette, relaxing, comforting and enjoyable and linear decreases for unpleasant, dislike taste and dislike cigarette over days ($P_s < 0.05$).

Several items also revealed a group \times cigarette interaction. Group differences (Nic > Denic) were greater

Table 1 Mean (SEM) ratings of cigarette characteristics during the controlled puffing procedure.

	<i>Denic</i>			<i>Nic</i>		
	<i>Day 3</i>	<i>Day 8</i>	<i>Day 13</i>	<i>Day 3</i>	<i>Day 8</i>	<i>Day 13</i>
Like cigarette	25.9 (7.7)	36.4 (10.5)	31.8 (9.1)	34.3 (7.1)	50.5 (9.3)	64.9 (9.4)
Like taste	30.3 (7.5)	39.6 (9.1)	28.4 (7.2)	33.6 (6.4)	38.0 (6.2)	56.5 (10.0)
Pleasant	34.1 (8.0)	34.4 (6.4)	39.0 (7.3)	46.1 (8.0)	52.7 (10.9)	64.5 (10.2)
Pleasurable excitement	26.5 (6.3)	27.8 (9.0)	24.6 (6.9)	39.4 (8.0)	39.1 (9.4)	50.2 (10.2)
Enjoyable	30.6 (7.7)	30.9 (9.3)	24.9 (7.6)	35.4 (6.8)	44.5 (9.9)	61.8 (10.7)
Sense of wellbeing	35.1 (8.0)	39.4 (8.5)	32.0 (8.4)	45.6 (10.2)	27.8 (7.6)	41.6 (10.6)
Exhilarating	24.7 (5.7)	28.2 (8.6)	22.3 (6.2)	29.4 (8.5)	24.7 (6.8)	40.7 (8.7)
Calming	36.6 (8.5)	34.4 (6.9)	31.8 (7.6)	45.3 (7.2)	38.2 (8.1)	50.0 (8.8)
Easier to concentrate	35.1 (8.0)	32.5 (6.8)	31.1 (7.0)	31.9 (7.5)	39.8 (8.6)	48.0 (9.4)
Relaxing	31.9 (7.3)	38.3 (8.8)	33.3 (6.9)	41.6 (7.7)	37.7 (7.8)	51.5 (7.6)
Comforting	34.4 (8.2)	38.3 (9.4)	33.5 (7.1)	38.1 (9.2)	40.0 (8.5)	53.5 (7.7)
High in nicotine	26.3 (5.1)	37.5 (8.1)	37.7 (7.7)	40.3 (8.7)	38.2 (8.9)	31.5 (9.4)
Dislike cigarette	69.6 (8.8)	58.5 (10.7)	64.8 (9.4)	48.9 (9.7)	44.4 (10.8)	33.1 (9.5)
Dislike taste	52.7 (10.9)	57.0 (10.5)	67.4 (9.0)	50.2 (11.0)	53.5 (8.8)	40.6 (11.2)
Harsh	37.0 (7.7)	45.6 (9.2)	46.6 (8.3)	39.1 (11.5)	26.3 (9.6)	21.8 (8.0)
Nauseating	18.9 (6.1)	18.1 (7.1)	28.8 (9.1)	6.0 (2.1)	11.7 (7.4)	13.4 (7.0)
Like drug effect	28.6 (5.9)	28.8 (7.8)	28.0 (7.1)	21.3 (6.3)	32.5 (9.5)	45.1 (8.5)

after the third compared to the first cigarettes of the day for relaxing ($P = 0.007$). Ratings of sense of wellbeing, enjoyable and calming showed a similar pattern; however, the third cigarette resulted in greater group differences only during the first few days of the study, after which group differences were similar across cigarettes (group \times cigarette \times linear day interaction: P s < 0.05). There was also a group \times cigarette interaction for dizziness ($P = 0.027$) and light-headedness ($P = 0.011$). In these cases, ratings were similar after the first cigarette but decreased significantly between the first and third cigarettes in the Nic (P s < 0.01) but not the Denic group. Adjusting for baseline generally confirmed the results presented above.

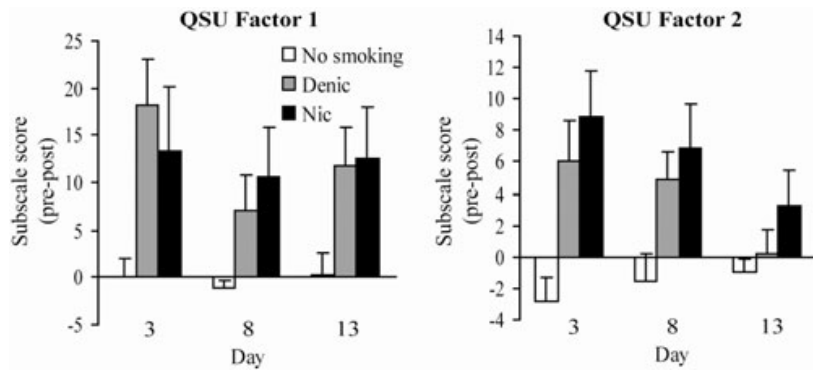
Group differences in ratings of cigarette effects after the controlled puffing procedure were similar to those observed during unrestricted smoking (Table 1). Smoking nicotine cigarettes tended to produce fewer negative effects and more positive effects than de-nicotinized cigarettes; some of these differences emerged or became exaggerated over days. There were non-significant trends (P s < 0.10) for higher ratings of pleasant, like cigarette, pleasurable excitement and enjoyable in the Nic condition, a significant interaction between group and day for like taste ($F_{2,36} = 3.31$, $P < 0.05$) and non-significant trends for group \times day interactions for sense of wellbeing, exhilarating and enjoyable (P s < 0.10). Conversely, ratings of dislike cigarette were significantly lower overall in the Nic group ($F_{1,18} = 4.62$, $P < 0.05$). Pairwise comparisons of responding on each of the test days indicated signifi-

cantly greater ratings of pleasant, like taste, calming, easier to concentrate, like cigarette ($d = 1.14$), relaxing, comforting, exhilarating, pleasurable excitement and enjoyable ($d = 1.26$) in the Nic than in the Denic group on day 13 ($P < 0.05$). In contrast, the Denic group exhibited significantly greater ratings of dislike taste, harsh, dislike cigarette ($d = 1.06$) and nauseating compared to the Nic condition on day 13 ($P < 0.05$). Significant pairwise comparisons were restricted entirely to day 13. For most of the 'positive' effects (e.g. like taste, like drug effect, like cigarette, easier to concentrate, enjoyable), ratings changed little in the Denic group over time, but increased significantly from day 3 to day 13 in the Nic group. Notably, there were no significant group differences in ratings of high in nicotine.

Craving and withdrawal

Preliminary analyses of background craving and withdrawal taken at 2 p.m. on unrestricted smoking days did not reveal any baseline (day 2) differences on the QSU or the SJWS. After randomization, factor 2 scores in the no smoking group tended to be higher than the Denic condition ($P = 0.084$) and were significantly higher than the Nic condition ($P = 0.028$), but did not differ between the smoking groups. No significant overall main effects of group were found for factor 1 of the QSU or any of the subscales of the SJWS. Pairwise comparison revealed a trend for greater SJWS craving in the no smoking group compared to both the Denic ($P = 0.069$) and Nic ($P = 0.092$) conditions, but no differences between the

Figure 4 Mean (\pm SEM) change (pre/post smoking) in factors 1 and 2 subscale scores of the QSU during the controlled puffing procedure on days 3, 8 and 13. Data are presented as change scores for clarity. Analyses were conducted on the raw data and are reported in detail in the text. Briefly, analyses revealed significant differences in the change from pre- to post-smoking for both the Denic and Nic conditions compared to no smoking ($P < 0.05$). No differences were found between the two smoking conditions



two smoking conditions. Analysis of the data after adjusting for baseline revealed a similar pattern of effects.

Craving was also assessed prior to and after the first and third cigarettes each day as measured by the Schuh–Stitzer VAS. These data were not related significantly to group at baseline. After randomization, all differences between the smoking groups were modulated by day. Specifically, there was a linear day \times group interaction for ‘How pleasant would a cigarette be right now?’, ‘How much do you want to smoke right now?’ and ‘How much do you crave a cigarette right now?’ (P s < 0.05) and a quadratic day \times group interaction for ‘How much do you want to smoke right now?’ and ‘How much do you crave a cigarette right now?’ (P s < 0.05). Each of these measures indicated that craving decreased slightly more over days in the Denic than the Nic group; however, this magnitude of these differences was small. More importantly, the groups did not differ in the degree to which smoking acutely suppressed craving (i.e. pre/post). There was a significant decrease in all five items of the Schuh–Stitzer after smoking, but this effect was not modulated by group and did not change significantly over days.

The QSU data also revealed suppression of craving after controlled puffing of both types of cigarettes, but little difference in the magnitude of craving suppression. Both nicotine and de-nicotinized cigarettes reduced factor 1 and factor 2 scores compared to not smoking (P s < 0.01) and the magnitude of craving suppression did not differ between the smoking groups (Fig. 4).

The SJWS administered during the controlled puffing sessions revealed significant effects of group on craving, physical symptoms and appetite. As with the QSU, smoking in the Nic or Denic groups decreased ratings of craving on the SJWS to a similar degree, both producing a significant decrease relative to not smoking (P s < 0.01). Disruptions in appetite were smaller in the no smoking and Denic conditions compared to the Nic group (P s < 0.05). However, close inspection of the data revealed that the smoking groups differed prior to smoking on day 3, suggesting that group differences were not a result of the cigarette assignment, but pre-existing

differences. Finally, there was a significant decline in physical symptoms after smoking nicotine ($P = 0.048$) but not de-nicotinized cigarettes compared to not smoking. Direct comparison of the two smoking groups, however, failed to reveal any significant differences in physical symptoms.

Estimates of nicotine content

Estimates of nicotine content immediately prior to discharge did not differ significantly between the two smoking conditions, although there was a trend for ratings of nicotine content to be higher in the Nic condition ($P = 0.099$).

Mood measures

There were no reliable differences between groups on the POMS or PANAS. Close inspection of the no smoking and Denic conditions indicated a trend for an increase in POMS-tension, POMS-depression and POMS-total mood disturbance compared to both baseline and the Nic condition, but these differences were small and failed to reach statistical significance. There were no reliable differences in sleep quantity or quality.

Physiological measures

Smoking nicotine-containing cigarettes resulted in a greater increase in heart rate than either smoking de-nicotinized cigarettes ($P = 0.006$) or not smoking ($P < 0.001$). Heart rate also increased across days more in the Nic than the no smoking group ($P = 0.019$). Interestingly, comparison of the no smoking and Denic conditions revealed a group \times pre/post interaction such that de-nicotinized cigarettes produced a small but significant increase in heart rate compared to not smoking ($P = 0.014$). There were no reliable group differences in blood pressure.

DISCUSSION

The exclusive, repeated availability of research cigarettes either containing or not containing nicotine differentially

influenced smoking behavior, the motivation to smoke and the subjective effects of smoking. Throughout the study, participants assigned to the Nic group smoked at levels that were similar to or higher than baseline rates of preferred brand smoking, increased their puff volume and showed no change in the motivation to smoke over repeated progressive ratio tests. Puff volume also increased in participants in the Denic group; however, both their rate of smoking and their motivation to smoke declined over time. Switching from the preferred brand to either novel research cigarette produced an immediate decrease in positive and increase in negative subjective effects regardless of nicotine content. Subsequent ratings changed little over repeated exposure in the Denic group; on parallel measures (e.g. like/dislike cigarette), negative ratings exceeded positive ratings of the assigned cigarettes throughout the study. In contrast, positive ratings of the nicotine-containing cigarettes increased and negative ratings declined with repeated exposure. From the second day of use onwards, participants in the Nic condition reported an overall liking for their assigned cigarettes (i.e. liking > disliking). Notably, the nicotine content of cigarettes had little effect on the suppression of craving by smoking. De-nicotinized cigarettes continued to suppress craving acutely even after 8 days of access.

It is important to note that partial, not complete, extinction of the reinforcing effects of smoking was observed in the present study. While the number of cigarettes smoked during unrestricted access and puffs earned during the progressive ratio session decreased over days in participants smoking de-nicotinized cigarettes, most participants (80%) continued to smoke. Consistent with the present findings, Buchhalter *et al.* [14] also observed a small (non-significant) reduction in smoking over a 5-day out-patient period as assessed by cigarette butts returned. Whether longer periods of exposure would lead to more complete extinction is unknown.

In contrast to the behavioral data, we found no evidence of extinction of the subjective effects of smoking. These data diverge from those reported by Rose & Behm [15]. In that study, the subjective rewarding effects of usual brand smoking were reduced after 2 weeks of exposure to de-nicotinized cigarettes. However, numerous differences make comparison of these studies difficult. Participants in the Rose & Behm study did not smoke de-nicotinized cigarettes exclusively during the 2-week exposure period and change in the subjective effects of the de-nicotinized cigarettes themselves was not assessed. Relatedly, our failure to observe a similar decrease in the positive subjective effects of smoking could be due to a floor effect. Positive subjective ratings were relatively low throughout the present study in participants smoking de-nicotinized cigarettes; in contrast, ratings of usual

brand cigarettes in the Rose & Behm study were substantially higher, leaving adequate room to observe a decrease.

The efficacy of de-nicotinized cigarettes to suppress craving was indistinguishable from nicotine-containing cigarettes throughout the study. This observation suggests that craving-suppression may be a particularly robust effect of de-nicotinized cigarettes that is less sensitive to extinction procedures. Consequently, relief from craving may be an important mechanism underlying continued smoking of de-nicotinized cigarettes (i.e. negative reinforcement). Whether even longer periods of exposure would lead to extinction of the craving-suppressing effects of smoking may have implications for the use of smoking stimuli in therapeutic settings.

Withdrawal symptoms other than craving tended to be elevated in both the no smoking and Denic conditions compared to baseline and the Nic condition, but these differences failed to reach significance; therefore, it was difficult to determine whether de-nicotinized cigarettes suppressed withdrawal [14] and whether withdrawal-suppression changed over days. The failure to observe significant withdrawal may have resulted from limited statistical power ($n = 10$ per group) and/or our use of the SJWS. Previous residential studies with larger sample sizes, within-subject designs and utilizing different scales have demonstrated reliable nicotine withdrawal [25,26], suggesting that future studies can effectively address this issue.

There is little experimental evidence supporting the widely held assumption that smoking stimuli acquire their effects through classical conditioning. The present study lends support to this hypothesis by demonstrating that smoking stimuli partly lose their reinforcing effects in the absence of nicotine (i.e. extinction) and that new stimuli acquire similar effects after being paired with nicotine. However, at least two additional hypotheses must also be considered. First, nicotine may contribute to smoking through its sensory effects [27]. Contrary to this hypothesis, the two smoking conditions reported similar initial ratings of taste which diverged only after repeated exposure; this finding is more consistent with learning a new association between taste and nicotine delivery in the Nic condition than stimulus degradation. Another possibility is that nicotine has other actions on the central nervous system that contribute to smoking behavior. Recent animal research has suggested that nicotine can non-associatively enhance reinforcement obtained from both conditioned [28] and unconditioned [29–32] reinforcers. Therefore, nicotine-containing cigarettes may maintain a higher level of smoking not because of nicotine's primary reinforcing effects but because nicotine directly enhances the conditioned reinforcing effects of sensorimotor smoking stimuli or other reinforcers in the

environment. Additional studies are needed to address this hypothesis directly.

Several limitations are worth noting. First, the use of an in-patient design limits generalizability because the determinants of smoking may differ between in-patient and naturalistic settings. In addition, the population sampled is more likely to be unemployed and/or from a lower socio-economic bracket than many smokers. Secondly, participants had moderate levels of dependence; whether these results would generalize to individuals of lower or higher levels of dependence is a question we intend to address in future studies. Finally, we assume that changes observed over days were a function of exposure to the smoking condition. However, change could also reflect either practice or learning effects, as time is confounded with repeated testing in the present design, or a change in the drive to smoke as time since initial nicotine abstinence elapses and withdrawal begins to subside in the Denic group.

In summary, repeated exposure to de-nicotinized cigarettes resulted in a gradual and incomplete reduction in the number of cigarettes smoked and in the motivation to smoke, but little change in their ability to suppress craving. This profile of effects highlight the important impact of non-nicotine sensorimotor stimuli as a determinant of smoking behavior and suggests that extinction of these effects in the absence of smoking does occur but may be slow. While the present study examined the longest duration of exposure to de-nicotinized cigarette smoking to date, additional research will be needed to follow these effects over even longer periods of time.

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