

22nd Century Limited, LLC

IND 103589

September 27, 2013

Bob Rappaport, M.D., Director
Food and Drug Administration
Center for Drug Evaluation and Research
Division of Anesthesia, Analgesia, and
Addiction Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

Re: IND 103589 (SN005) X-22 Smoking Cessation Product
Annual Report

Dear Dr. Rappaport,

Reference is made to our Initial IND 103,589 for X-22 Smoking Cessation Product, submitted June 21, 2011 (SN0000), for our investigational drug X-22 Smoking Cessation Product for the indication of smoking cessation.

We are submitting herewith the Annual Report for the period September 2, 2012 to September 1, 2013 for this IND.

If you have additional questions or require further information, please do not hesitate to contact me by e-mail at (b) (4), (b) (6) or by telephone at (b) (6), (b) (4)

Kind regards,

(b) (4), (b) (6)
Regulatory Affairs Consultant

9530 Main Street, Clarence, New York 14031 • TEL 716-270-1523 • FAX 716-877-3064 •
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

INVESTIGATIONAL NEW DRUG APPLICATION (IND)

(Title 21, Code of Federal Regulations (CFR) Part 312)

Form Approved: OMB No. 0910-0014
Expiration Date: April 30, 2015
See PRA Statement on page 3.

NOTE: No drug/biologic may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40)

1. Name of Sponsor
22nd Century Limited, LLC

2. Date of Submission *(mm/dd/yyyy)*
09/27/2013

3. Sponsor Address

Address 1 *(Street address, P.O. box, company name c/o)*
9530 Main Street

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City
Clarence

State/Province/Region
NY

Country
USA

ZIP or Postal Code
14031

4. Telephone Number *(Include country code if applicable and area code)*
(b) (6)

5. Name(s) of Drug *(Include all available names: Trade, Generic, Chemical, or Code)*
X-22 Smoking Cessation Product

Continuation Page for #5

6. IND Number *(If previously assigned)*
103589

7. (Proposed) Indication for Use
Smoking cessation

Is this indication for a rare disease (prevalence <200,000 in U.S.)? ☐ Yes ☐ No

Does this product have an FDA Orphan Designation for this indication? ☐ Yes ☑ No

If yes, provide the Orphan Designation number for this indication:

Continuation Page for #7

8. Phase(s) of Clinical Investigation to be conducted ☐ Phase 1 ☑ Phase 2 ☐ Phase 3 ☐ Other *(Specify)*:

9. List numbers of all Investigational New Drug Applications (21 CFR Part 312), New Drug Applications (21 CFR Part 314) , Drug Master Files (21 CFR Part 314.420) , and Biologics License Applications (21 CFR Part 601) referred to in this application.

10. IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 0000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 0001." Subsequent submissions should be numbered consecutively in the order in which they are submitted..

Serial Number
0005

11. This submission contains the following *(Select all that apply)*

☐ Initial Investigational New Drug Application (IND)
☐ Request For Reactivation Or Reinstatement
☐ Development Safety Update Report (DSUR)
☐ Response to Clinical Hold
☑ Annual Report
☐ Other *(Specify)*:
☐ Response To FDA Request For Information
☐ General Correspondence

Protocol Amendment(s)
☐ New Protocol
☐ Change in Protocol
☐ New Investigator
☐ PMR/PMC Protocol

Information Amendment(s)
☐ Chemistry/Microbiology
☐ Pharmacology/Toxicology
☐ Clinical ☐ Statistics
☐ Clinical Pharmacology

Request for
☐ Meeting
☐ Proprietary Name Review
☐ Special Protocol Assessment
☐ Formal Dispute Resolution

IND Safety Report(s)
☐ Initial Written Report
☐ Follow-up to a Written Report

12. Select the following only if applicable. *(Justification statement must be submitted with application for any items selected below. Refer to the cited CFR section for further information.)*

Expanded Access Use, 21 CFR 312.300

☐ Emergency Research Exception From Informed Consent Requirements, 21 CFR 312.23 (f)
☐ Charge Request, 21 CFR 312.8
☐ Individual Patient, Non-Emergency 21 CFR 312.310
☐ Individual Patient, Emergency 21 CFR 312.310(d)
☐ Intermediate Size Patient Population, 21 CFR 312.315
☐ Treatment IND or Protocol, 21 CFR 312.320

For FDA Use Only

CBER/DCC Receipt Stamp	DDR Receipt Stamp	Division Assignment
		IND Number Assigned

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13. Contents of Application – This application contains the following items *(Select all that apply)*

- [x] 1. Form FDA 1571 *(21 CFR 312.23(a)(1))*
- [x] 2. Table of Contents *(21 CFR 312.23(a)(2))*
- [x] 3. Introductory statement *(21 CFR 312.23(a)(3))*
- [x] 4. General Investigational plan *(21 CFR 312.23(a)(3))*
- [ ] 5. Investigator's brochure *(21 CFR 312.23(a)(5))*
- [ ] 6. Protocol(s) *(21 CFR 312.23(a)(6))*
  - [ ] a. Study protocol(s) *(21 CFR 312.23(a)(6))*
  - [ ] b. Investigator data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
  - [ ] c. Facilities data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
- 6. Protocol(s) *(Continued)*
  - [ ] d. Institutional Review Board data (21 CFR 312.23(a)(6)(iii)(b)) or completed Form(s) FDA 1572
- [x] 7. Chemistry, manufacturing, and control data *(21 CFR 312.23(a)(7))*
  - [ ] Environmental assessment or claim for exclusion *(21 CFR 312.23(a)(7)(iv)(e))*
- [x] 8. Pharmacology and toxicology data (21 CFR 312.23(a)(8))
- [x] 9. Previous human experience *(21 CFR 312.23(a)(9))*
- [ ] 10. Additional information *(21 CFR 312.23(a)(10))*
- [ ] 11. Biosimilar User Fee Cover Sheet *(Form FDA 3792)*
- [ ] 12. Clinical Trials Certification of Compliance *(Form FDA 3674)*

14. Is any part of the clinical study to be conducted by a contract research organization? [ ] Yes [x] No

If Yes, will any sponsor obligations be transferred to the contract research organization? [ ] Yes [ ] No

If Yes, provide a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred *(use continuation page)*. Continuation Page for #14

15. Name and Title of the person responsible for monitoring the conduct and progress of the clinical investigations

Michael R. Moynihan, Ph.D., VP Research and Development, 22nd Century Limited, LLC

16. Name(s) and Title(s) of the person(s) responsible for review and evaluation of information relevant to the safety of the drug

(b) (4), (b) (6) Medical Monitor

(b) (4) [redacted]

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold or financial hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set forth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

17. Name of Sponsor or Sponsor's Authorized Representative

(b) (4), (b) (6)

18. Telephone Number *(Include country code if applicable and area code)*

(b) (4), (b) (6)

19. Facsimile (FAX) Number *(Include country code if applicable and area code)*

20. Address

Address 1 *(Street address, P.O. box, company name c/o)*

(b) (4), (b) (6)

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City

(b) (4), (b) (6)

State/Province/Region

(b) (4), (b) (6)

Country

USA

ZIP or Postal Code

(b) (4), (b) (6)

21. Email Address

(b) (6), (b) (4) [redacted]

22. Date of Sponsor's Signature *(mm/dd/yyyy)*

09/27/2013

23. Name of Countersigner

24. Address of Countersigner

Address 1 *(Street address, P.O. box, company name c/o)*

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City

State/Province/Region

Country

United States of America

ZIP or Postal Code

WARNING : A willfully false statement is a criminal offense (U.S.C. Title 18, Sec. 1001).

25. Signature of Sponsor or Sponsor's Authorized Representative

(b) (4), (b) (6)

Sign

26. Signature of Countersigner

Sign

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2013 IND ANNUAL REPORT

IND No.:	103,589
Serial No.:	0005
Product Name:	X-22 Smoking Cessation Product
Indication:	Smoking Cessation
Reporting Period:	02 September 2012 to 01 September 2013
Date of Report:	27 Sep 2013

CONFIDENTIAL

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1 INDIVIDUAL STUDY INFORMATION

There were no clinical studies of X-22 Smoking Cessation Product (X-22) active during the reporting period.

1.1 Studies in Progress

No trials of the X-22 Smoking Cessation Product sponsored by 22nd Century Limited, LLC were being conducted during the past year.

1.2 Completed Studies

No trials of the X-22 Smoking Cessation Product sponsored by 22nd Century Limited, LLC were completed during the past year.

One clinical study was completed during the prior reporting period, Study X-22-201 (Table 1). The clinical study report is in preparation and will be submitted to the FDA when available.

Table 1 IND Studies

Protocol Number	Title	No. Planned/ Entered	Demographics			No. Completed/ Discontinued	Study Status
			Subject Age Range (years)	Gender	Race		
X-22-201	A Prospective, Double-blind, Randomized, Active Controlled, Parallel Group, Multicenter Phase II Clinical Trial to Evaluate the Effectiveness of X-22 as a Smoking Cessation Aid	216/234	20-65	128 M 106 F	169 W 59 B 3 A 3 O	175/59	Completed

Abbreviations: A=Asian, B=Black, F=female, M=male, O=other, W=white
Source: X-22-201 draft clinical study report Table 14.1.1.2

1.3 Foreign or other Clinical Trials Conducted by 22nd Century Limited, LLC Not Conducted Under IND 103,589.

There are no clinical trials of X-22 Smoking Cessation Product being conducted outside of the United States.

1.4 Non-22nd Century Limited, LLC Sponsored Studies

There are no clinical trials of the X-22 Smoking Cessation Product being conducted by sponsors other than 22nd Century Limited, LLC.

1.5 Non-22nd Century Limited, LLC Sponsored Studies

A number of independent clinical studies have been carried out recently with other Very Low Nicotine (VLN) cigarettes. These include studies with Quest®, Xodus®, and Spectrum® cigarettes.

1.5.1 Studies of Smoking Cessation

Two studies mentioned as ongoing in the previous annual report that used Quest® or Xodus® VLN cigarettes were completed. Results of one of these studies were published.

1.5.1.1 A Study of VLN cigarettes, Nicotine Patch, and VLN cigarettes plus Nicotine Patch

Hatsukami *et al*. evaluated the effect of six weeks treatment with combined use of a 21 mg nicotine patch and VLN cigarettes compared to use of VLN cigarettes alone or 21 mg nicotine patch alone on abstinence, withdrawal, smoking behavior, and toxicant exposure in randomized, parallel arm study involving 219 subjects.[1] All groups received six weeks of additional behavioral treatment with follow-ups up to six months.

Subjects enrolled early in the trial received Quest 3® cigarettes as the VLN cigarette. After 27% of the subjects were enrolled, those assigned to VLN cigarette conditions received Xodus® cigarettes rather than Quest 3® cigarettes, which were no longer available. The results for those receiving Xodus® cigarettes only were similar to the results for all subjects receiving Quest 3® cigarettes

There were no significant differences across the three treatment groups for continuous abstinence from the end of treatment (week 6) or 7 day point prevalence abstinence at any time point. Continuous abstinence rates at week 12 (6 weeks post-treatment) were 10.5% to 13.9%, at week 24, 7.9% to 11.4%, and at week 36, 7.9% to 10.1%. CO and cotinine verified 7 day point prevalence abstinence rates were 23.7% to 24.1% at week 12, 17.1% to 20.0% at week 24, and 17.7% to 19.7% at week 36.

CO and Cotinine Verified Continuous Abstinence

	Treatments						
	VLNC cigarette (n=79)		*Nicotine Patch (n=80)*		*VLNC + NP (n=76)*		
Week	*# abstinent*	*%*	*# abstinent*	*%*	*# abstinent*	*%*	*p-value*
12	11	13.9	11	13.8	8	10.5	0.776
24	9	11.4	10	12.5	6	7.9	0.625
36	8	10.1	8	10.0	6	7.9	0.867

CO Verified Point Prevalence Abstinence

	Treatments						
	VLNC cigarette (n=79)		*Nicotine Patch (n=80)*		*Combination (n=76)*		
Week	*# abstinent*	*%*	*# abstinent*	*%*	*# abstinent*	*%*	*p-value*
12	21	26.6	24	30.0	22	29.0	0.888
16	21	26.6	20	25.0	23	30.3	0.752
24	18	22.8	17	21.3	16	21.1	0.959
36	15	19.0	19	23.8	16	21.1	0.763

CO and Cotinine Verified Point Prevalence Abstinence

	Treatments						
	VLNC cigarette (n=79)		*Nicotine Patch (n=80)*		*Combination (n=76)*		
Week	*# abstinent*	*%*	*# abstinent*	*%*	*# abstinent*	*%*	*p-value*
12	19	24.1	19	23.8	18	23.7	0.998
24	15	19.0	16	20.0	13	17.1	0.896
36	14	17.7	15	18.8	15	19.7	0.950

Table 3 from Hatsukami, *et al.* 2013

The median time to relapse since treatment onset was longer for the VLN cigarette + nicotine patch condition, 7.1 weeks (95% CI 6.7 to 7.7), compared to the VLN cigarette alone condition, 2.6 weeks (CI 1.7 to 5.9), and nicotine patch alone condition 2.1 weeks (CI 1.6 to 3.9) weeks.

No significant differences were observed between treatments for craving. Subjects assigned to VLN cigarettes + nicotine patch had significantly lower withdrawal symptoms than those in the nicotine patch alone group ($p = 0.008$), but not significantly lower than those in the VLN cigarette alone group ($p = 0.092$).

In both groups receiving VLN cigarettes, the number of cigarettes per day declined from baseline to week 6, with subjects assigned to the VLN cigarette + nicotine patch showing a greater decline, from a baseline of 17.7 ± 6.3 to 11.4 ± 7.6 cigarettes per day, compared to a decline from 19.5 ± 8.6 to 16.2 ± 10.2 in the VLN cigarette only group.

1.5.2 Other Clinical Studies Using VLN Cigarettes

A number or other clinical studies using VLN cigarettes have been carried out or are in progress using Spectrum® or Quest 3® cigarettes. The studies summarized below did not directly investigate the use of VLN cigarettes as a tool for smoking cessation.

22nd Century is providing the National Institute on Drug Abuse (NIDA) with SPECTRUM® research cigarettes that have eight different levels of nicotine content in the tobacco filler (from 0.4 to 25 mg/g) for ongoing research on how different nicotine levels in cigarettes affect smoking behavior and the results of smoking. The two lowest nicotine levels, 0.4 mg/g and 1.3 mg/g, are

similar to the nicotine concentrations in VLN cigarettes that have been used in clinical studies to date.

Although Quest 3® VLN cigarettes have not been sold by the manufacturer since 2008, results of a number of additional clinical studies using Quest 3® have been reported.

1.5.2.1 Dose–Response Effects of Spectrum® Research Cigarettes

Initial studies of Spectrum® cigarettes with three different nicotine levels (VLN cigarettes with a nicotine concentration of 0.4 mg/g, as well as cigarettes with nicotine concentrations of 6 mg/g and 12 mg/g) were carried out by the University of Minnesota, University of Pittsburgh and NIDA.[2]

In the first study, 51 subjects each smoked 4 puffs on each of four cigarettes (usual brand, followed by Spectrum® cigarettes with 0.4, 6 and 12 mg/g nicotine presented in random order) in a single session, with puffing bouts separated by 30 minute intervals. Menthol and non-menthol cigarettes smokers were assigned their respective preference.

Subjects took four puffs (1 bout) on each cigarette type at 30 second interpuff intervals. Heart rate and blood pressure were measured immediately after each puffing bout. Alveolar carbon monoxide (CO) level was measured 2 min after each bout. Subjects then completed subjective rating scales.

Smokers rated the cigarettes with 0.4 mg/g nicotine lower than the 6 mg/g and 12 mg/g cigarettes and usual brand for satisfaction, psychological reward, enjoyment of respiratory tract sensation, and craving reduction.

Table 1. Study 1: Subscales of the Modified Cigarette Evaluation Questionnaire (mCEQ) by Nicotine Level

Measurement[a] (0–100 Scale)	Usual Brand Mean (*SE*)	Lower LS Means (SE)[b]	Intermediate LS Mean (*SE*)[b]	Higher LS Mean (*SE*)[b]	Lower vs. Intermediate	Lower vs. Higher	Intermediate vs. Higher
Satisfaction	83.6 (1.4)	36.8 (4.1)	54.1 (4.1)	61.4 (4.1)	$\|t\| = 3.26$*	$\|t\| = 4.65$***	$\|t\| = 1.38$
Psychological Reward	53.7 (2.2)	33.5 (3.1)	40.9 (3.1)	43.6 (3.1)	$\|t\| = 1.85$	$\|t\| = 2.53$+	$\|t\| = 0.68$
Aversion	13.0 (1.7)	16.7 (2.8)	14.1 (2.8)	18.2 (2.8)	$\|t\| = 0.73$	$\|t\| = 0.40$	$\|t\| = 1.14$
Enjoyment of Sensation	73.1 (2.4)	32.7 (4.2)	49.3 (4.2)	54.8 (4.2)	$\|t\| = 2.70$*	$\|t\| = 4.09$**	$\|t\| = 1.03$
Craving Reduction	62.8 (3.3)	42.8 (4.5)	54.9 (4.5)	58.6 (4.5)	$\|t\| = 2.07$	$\|t\| = 2.71$+	$\|t\| = 0.64$

Notes. LS = least square; SE = standard error.
[a]Subscale scores were averaged across the items.
[b]Adjusted for baseline response (usual brand), gender, menthol use, study site, cigarette order, interactions between nicotine level and menthol and gender, and repeated measures across subjects.
+$p \le .05$. *$p \le .01$. **$p \le .001$. ***$p \le .0001$.

From Hatsukami *et al*., Nicotine Tob Res. 2013; 15(6):1113-1121

The 0.4 mg/g nicotine cigarettes were also associated with a significantly lower monetary value than the 6 mg/g or 12 mg/g cigarettes when subjects were asked the price at which they would prefer money over a pack of cigarettes, $3.44 ± 0.41 vs. $4.90 ± 0.40 and $4.88 ± 0.40 (p = 0.020 for 6 vs. 0.4 mg/g and p = 0.019 for 12 vs. 0.4 mg/g cigarettes). There was a significant interaction with gender; with larger differences in price by nicotine content among women, $2.88 ± 0.66 for the 0.4 mg/g nicotine cigarette vs. $5.68 ± 0.66 and $5.01 ± 0.66 for the 6 and 12 mg/g

cigarettes, while among men the differences in prices of $4.00 ± 0.47 for the 0.4 mg/g nicotine cigarette, $4.15 ± 0.47 for the 6 mg/g cigarette, and $4.88 ± 0.46 for the 12 mg/g cigarette were not smaller and statistically significant.

The second study used a between-subject design in which 36 subjects smoked their usual brand during a 1 week baseline period and then were randomly assigned to 1 of the 3 cigarettes for 1 week.

Subjects assigned to the VLN cigarettes smoked significantly fewer study cigarettes over the course of the treatment week than those assigned to the 12 mg/g nicotine cigarettes, 92.1 ± 15.9 vs. 157.6 ± 15.2, ($p = 0.018$), and fewer study cigarettes compared with usual brand cigarettes smoked during the baseline period, although that difference was not statistically significant (change: −10.6 ± 14.0 cigarettes/week, $p = 0.454$).

Subjects were instructed to smoke the study cigarette exclusively during the second week, but were told to also record the number of any non-study cigarettes smoked each day. Five subjects in the 0.4 mg/g group, 4 in the 6 mg/g group, and 2 in the 12 mg/g group reported smoking non-study cigarettes during the second week.

Subjects in the 0.4 mg/g nicotine cigarette group had a significant decrease in exhaled CO level compared to baseline, from 17.1 ± 1.4 to 11.3 ± 2.3, whereas CO did not change significantly from baseline in the 6 mg/g and 12 mg/g nicotine conditions.

1.5.2.2 Comparison of the Effects of Nicotine Content on Smoking Behavior in Smokers Who Do Not Intend to Quit

A study led by Eric Donny at the University of Pittsburgh is comparing the effects of cigarettes with different nicotine concentrations on smoking behavior in over a period of 6 weeks in a study with 840 subjects.[3] Smokers that are not currently using or seeking smoking cessation treatment are randomized to one of seven arms: usual brand, or Spectrum® cigarettes with machine smoking nicotine yields of 0.8, 0.3, 0.12, 0.07 or 0.03 mg/cigarette (filler nicotine concentrations of 16, 6, 2.5, 1.3, and 0.4 mg/g) and tar yields of 8-10 mg per cigarette, or a higher-yield style of cigarettes with the filler that has 0.4 mg/g nicotine with machine smoking yields of 0.04 mg nicotine and 13 mg tar per cigarette.

1.5.2.3 A Study of Effects of Smoking on Startle Reactivity among Smokers with PTSD

A study by Calhoun *et al*. examined effects of smoking on the acoustic startle response (ASR) in smokers with and without posttraumatic stress disorder (PTSD).[4] Smokers with PTSD report that smoking provides significant relief of negative affect and PTSD symptoms. Exaggerated startle response is a feature of PTSD. The objectives of the study were to examine baseline ASR in smokers with and without PTSD under conditions of overnight smoking abstinence, and evaluate the effects trauma versus neutral script presentations and smoking usual brand cigarette vs. Quest 3® VLN cigarettes on ASR. Forty eight smokers with and without PTSD were randomized in a 2 x 2 x 3 design (PTSD vs. non-PTSD; trauma vs. neutral script; usual brand vs. Quest 3® cigarette vs. no smoking).

PTSD participants demonstrated higher ASR compared to non-PTSD participants, and PTSD participants reported greater negative affect following a trauma-related script. PTSD participants following a trauma-related script demonstrated higher ASR in the usual brand smoking

condition, suggesting nicotine may actually potentiate or maintain an exaggerated startle response.

1.5.2.4 A Study of the Effects of Nicotine Dose and Nicotine Dose Expectancy on Smoking Outcomes

This study by Juliano *et al*. investigated the independent and interactive effects of nicotine dose and nicotine dose expectancy on smoking outcomes in 148 smokers using a 2 x 2 balanced placebo design (nicotine vs. placebo; told nicotine vs. told placebo).[5]

Smokers completed a Rapid Visual Information Processing Task (RVIP) and measures of smoking urge, mood, and cigarette ratings (e.g., satisfying) after smoking a single cigarette under one of four smoking conditions: (Quest 1® cigarette or Quest 3® cigarette) x (instructed cigarette contained nicotine or instructed cigarette did not contain nicotine).

Nicotine dose had effects. Quest 1® cigarettes produced better sustained attention performance than Quest 3® cigarettes as indicated by RVIP reaction time, hits, and sensitivity, and also produced better mood and greater rewarding subjective effects of the cigarettes on 11 of 11 dimensions.

Nicotine expectancy also had effects. Subjects told that the cigarette contained nicotine had fewer RVIP false alarms, and reported better mood and greater rewarding subjective effects of the cigarettes on 9 of 11 dimensions compared to those told that the cigarette was a placebo containing no nicotine.

Nicotine dose by nicotine dose expectancy interactions were observed. The dose expectancy manipulation produced differential effects for urge and tension-anxiety only among those who smoked placebo cigarettes. In contrast, the dose expectancy manipulation for self-reported vigor-activity produced effects only among those who smoked nicotine cigarettes.

1.5.2.5 A Study of the effect of Nicotine Level on Withdrawal and Smoking Behavior in Low and High Sensation Seekers.

A study by Lee *et al*. investigated the effects of cigarettes having three different nicotine levels (Quest 3® VLN cigarettes, Quest 1®, and Kent®) in twenty smokers with impulsive sensation-seeking subscale scores in the upper or lower quartile of the Impulsive Sensation-Seeking Scale of the Zuckerman–Kuhlman Personality Questionnaire.[6]

Self-report questionnaires, psychomotor and cognitive tasks, and cardiovascular measures were completed following *ad libitum* smoking on Day 1, following 24 hour tobacco deprivation on Day 2, and then following paced smoking of eight puffs from one experimental cigarette on Day 2. In addition, self-administration of additional experimental cigarettes with the same nicotine level was analyzed during a 2 hour period after the initial tobacco administration. All cigarettes were smoked using a mouthpiece connected to a volumetric transducer to record the duration and volume of each puff.

Tobacco deprivation resulted in expected withdrawal effects on self-report, performance, and cardiovascular measures, and tobacco smoking ameliorated many of these effects in a nicotine dose-dependent manner in both low and high sensation seekers. However, in high sensation seekers, deprivation effects on a small subset of measures (e.g., restlessness, elation) were

ameliorated by smoking independent of nicotine yield, whereas amelioration was dependent on nicotine yield among low sensation seekers. However, self-administration of cigarettes did not differ across nicotine level in low sensation seekers, while the number of cigarettes smoked, total puff volume, and puff duration were significantly higher for the highest nicotine cigarette in high sensation seekers only. The authors suggest that high sensation may obtain more withdrawal relief from the nonnicotine effects of smoking, but may also obtain more reinforcement from the higher nicotine levels during ad libitum smoking.

1.5.2.6 A Study of Effects of Mu Opioid Receptor Polymorphisms on Brain Receptor Binding Potential and Subjective Reward Following Smoking of Cigarettes Differing in Nicotine Content

A study by Ray *et al*. examined the effects of smoking Quest 3® VLN cigarettes or Quest 1® moderate nicotine content cigarettes on mu opioid receptor (MOR) and subjective responses.[7]

Twenty-two smokers prescreened for MOR genotype (12 A/A, 10 */G) completed [^{11}C]carfentanil positron emission tomography (PET) imaging to measure MOR availability during two separate sessions, both occurring after overnight (14 h) abstinence. Fifteen minutes before the scan in session 1, participants smoked a Quest 1® cigarette, and fifteen minutes before the scan in session 2, a Quest 3® VLN cigarette. Participants and data analysts were blind to the cigarette type. To standardize exposure, the cigarettes were smoked through a controlled puffing procedure (participants were allowed to take a total of six puffs after lighting the cigarette, each puff was separated by 30 s).

Smokers homozygous for the wild-type OPRM1 A allele exhibited significantly higher levels of MOR binding potential than smokers carrying the G allele in bilateral amygdala, left thalamus, and left anterior cingulate cortex regardless of the type of cigarette.

The higher nicotine cigarette was rated as significantly more rewarding than the VLN cigarette, and subjective reward did not differ by genotype. Among G allele carriers only, the extent of subjective reward difference (VLN versus moderate nicotine cigarette) was associated significantly with differences in MOR binding potential in right amygdala, caudate, anterior cingulate cortex, and thalamus.

1.5.2.7 Interaction of Nicotine Content of Cigarettes and Alcohol on the Rating of Attractiveness

A study by Attwood *et al*. examined the interaction of nicotine and alcohol on rating of attractiveness of faces and landscapes was examined.[8] Ninety-six male and female social alcohol consumers who were “light” smokers (no more than 14 cigarettes per week) were randomized to one of four conditions using a 2 x 2 factorial design, (alcoholic or non-alcoholic beverage) x (Quest 1® or Quest 3®), with equal numbers of male and female subjects in each condition.

Each subject participated in a single session. Abstinence was assessed by testing of breath alcohol and carbon monoxide. Subjects completed baseline measures of cigarette and alcohol craving and mood, were given 10 minutes to consume the beverage (alcoholic or placebo) followed by a 10 minute absorption period, and then were provided with a single cigarette (Quest 1® moderate nicotine cigarette or Quest 3® VLN cigarette) and lighter. After the subject had taken a puff of the cigarette the photographic images were presented on a computer and rated for

attractiveness using a 7-point Likert scale. On completion of the attractiveness rating task, subject completed measures of craving and mood, and were asked whether they believed their drink had contained alcohol and whether their cigarette had contained nicotine.

There was a significant positive effect of alcohol on ratings of attractiveness ($p = 0.031$) and a trend toward a main effect of cigarette type ($p = 0.057$). Nicotine and alcohol appeared to have additive effects on ratings of attractiveness, with the highest ratings in the alcoholic drink/higher nicotine cigarette condition.

There was a significant increase from baseline in self-reported intoxication score in all four conditions, with larger increases in response to alcohol, and the largest increase in the group who received alcoholic drink and higher nicotine cigarette.

Participants correctly identified drink content in 77% of cases, and cigarette content in 68% of cases, both of which differ significantly from chance ($p < 0.001$).

1.5.2.8 A Comparison of Effects of Smoking Usual Brand or VLN Cigarette on Withdrawal Symptoms in Smoking in Smokers With and Without Posttraumatic Stress Disorder

A study by Dedert *et al*. compared withdrawal symptoms and the effects of 3 smoking conditions, (continued abstinence, smoking a conventional cigarettes, and smoking Quest 3® VLN cigarettes) in smokers with (n = 17) or without (n = 30) posttraumatic stress disorder (PTSD).[9] Each subject completed three 60 minute experimental sessions on separate days following overnight smoking abstinence, in which the smoking conditions were assigned in random order. Data from this preliminary investigation is from a subset of subjects in the study investigating acoustic startle in smokers with and without PTSD described in Section 1.5.2.3. All subjects in this subset received neutral scripts in the script-driven imagery portion of that study.

Following overnight abstinence, the PTSD group was higher in craving ($p < 0.001$) and habit withdrawal ($p = 0.001$) relative to the non-PTSD group, and higher in urge to smoke in anticipation of pleasure ($p < 0.001$) and to relieve withdrawal/negative affect ($p = 0.014$) on the "Brief Questionnaire of Smoking Urges.

Overall, usual brand cigarettes were associated with a larger decrease in craving than VLN cigarettes ($p <0.001$), although the VLN cigarettes significantly reduced craving compared to the no-smoking condition ($p = 0.016$). Usual brand smoking provided a significantly greater effect than VLN cigarettes on habit withdrawal scores ($p < 0.001$), but the VLN cigarette smoking reduced habit withdrawal compared to no smoking ($p = 0.016$).

1.5.2.9 1.5.2.9 A Comparison of the Effects of Smoking Cigarettes or Different Nicotine Content on μ-Opioid Receptor Availability and Negative Affect

A study by Falcone *et al*. compared the effects of smoking Quest 1® or Quest 3® VLN cigarettes on μ-opioid receptor (MOR) availability in the amygdala and motivation to smoke for negative affect relief.[10] This study was a secondary analysis of data from the study by Ray *et al*. summarized in Section 1.5.2.6.

Twenty-two smokers were scanned on two separate occasions after overnight abstinence using [^{11}C]carfentanil positron emission tomography imaging after smoking a single Quest 1® moderate nicotine cigarette or Quest 3® VLN cigarette. Higher MOR availability in the amygdala

was associated with motivation to smoke to relieve negative affect. However, as was reported in the study of Ray *et al*., there was no difference in MOR availability between cigarette types, and neither cigarette produced significant changes in negative or positive mood.

1.5.2.10 A Study of Effect of Nicotine Response Expectancy and Nicotine Content on Cognitive Performance

Harrell and Juliano examined the effect of instructions that a cigarette would either enhance or impair cognitive and motor performance crossed with smoking cigarettes of different nicotine content in a 2 x 2 factorial study.[11] Eighty smokers were randomly assigned to one of four conditions: (a) told that nicotine enhances performance/given moderate nicotine cigarette (Quest 1®); (b) told that nicotine enhances performance/given VLN cigarette (Quest 3®); (c) told nicotine impairs performance/given moderate nicotine cigarette; and (d) told nicotine impairs performance/given VLN cigarette. All subjects were told the cigarettes contain "a fairly strong dose of nicotine."

Following a period of 12 h abstinence from smoking (confirmed by exhaled CO), subjects completed self reports of withdrawal symptoms and smoking urges and carried out a rapid visual information processing task and measure of psychomotor speed. Subjects were and then were given a single Quest 1® moderate nicotine cigarette or Quest 3® VLN cigarette to smoke ad libitum, and instructions intended to influence the expected effects of smoking. After smoking, participants again completed self-reports and performance measures, and exhaled CO was measured.

Compared to those "told impair," those "told enhance" reported more psychological reward, enjoyable physical sensations, and craving reduction from the cigarette, as well as greater motivation to perform well on a cognitive task, regardless of the type of cigarette received. The instructions, however, had no significant effect on performance on the Rapid Visual Information Processing Task.

Smoking a Quest 3® VLN cigarette produced reports of lower satisfaction, craving reduction and dizziness than smoking a Quest 1® cigarette. However, there was no difference in the ratings of smoking urge. The VLN cigarette group also had significantly lower performance on the Rapid Visual Information Processing Task, with a lower number of hits and lower sensitivity, which takes into account the number of both hits and false alarms.

1.5.2.11 A Comparison of Smoking Topography During Successive Smoking of Cigarettes with Different Nicotine Content

A study by MacQueen *et al*. investigated the effects of nicotine content on smoking topography (e.g., puff number, volume, duration, interpuff interval, and peak flow) over the course of smoking 4 successive cigarettes.[12]

Eighty-three smokers recruited for a study investigating of the effects of nicotine content on neural indices of attention completed two separate 2.5-h sessions scheduled 3 to 14 days apart. In one session in the subjects smoked four Quest 1®cigarettes containing 8.9 mg nicotine/cigarette (13 mg/g), and in the other, four Quest 3® VLN cigarettes containing 1.0 mg nicotine/cigarette (1.5 mg/g). Cigarette type was double-blinded and counterbalanced. Sixteen participants were

excluded from the analysis because of procedural errors in the use of the smoking topography equipment, leaving a sample of 67 participants.

Abstinence from smoking and alcohol for a minimum of 12 h prior to the sessions was required, and confirmed by measurement of exhaled CO (<10 ppm, or half the level at baseline) and blood alcohol (<0.001%).

Subjects smoked the four cigarettes at their own pace through a mouthpiece connected to a smoking topography device, with smoking of each subsequent cigarette initiated 40 min after initiation of the previous cigarette. Immediately after smoking a cigarette the Modified Cigarette Evaluation Questionnaire was completed. Participants were prepped with an electroencephalogram (EEG) cap between smoking bouts 1 and 2, and administered attention and working memory tasks between smoking bouts 2 and 3, and bouts 3 and 4 (according to this data is to be reported in a separate publication).

The smokers demonstrated compensatory behavior while smoking four VLN cigarettes, but these effects generally dissipated across cigarette trials. Puff volumes were significantly higher for the VLN cigarette during the first and second smoking bouts, but not during the remaining smoking bouts. A similar pattern was apparent for puff duration, except that puff duration was significantly higher during the first, second, and fourth smoking bout. Total inter-puff interval was significantly reduced for the VLN cigarette compared to Quest 1®. No significant difference between cigarette types was observed in number of puffs taken and average peak air velocity.

Smoking satisfaction and sensory feelings were significantly lower for Quest 3® than for Quest 1®. These subjective ratings also showed reductions across the smoking bouts.

Quest 3® reduced cravings to a lesser extent than Quest 1®, but craving reduction was attenuated in later cigarette bouts, and the difference between cigarette types was only significant during the first smoking bout ($p < 0.001$), approached significance second bout ($p = 0.05$), and was non-significant in later bouts.

1.5.2.12 A Comparison of the Effects of Nicotine Content on Subjective Ratings of Positive and Negative Reinforcement from Smoking

A study by Lindsey *et al*. examined effects of smoking conventional cigarettes (Marlboro® Red 100) or Quest 3® VLN cigarettes on subjective ratings of positive and negative reinforcement.[13] The cigarettes were smoked through an fMRI-compatible smoking device with concurrent fMRI scanning. A recent publication described changes in in subjective ratings of reinforcement over the time-courses of the smoking sessions. The authors stated that future analyses will attempt to link these changes in subjective ratings to neuroimaging measures of simultaneous regional hemodynamic changes in brain in order to better understand the neuronal substrates mediating the subjective effects of nicotine and placebo smoking.

Fourteen cigarette smokers each completed two fMRI scanning sessions on separate days. During one visit two Marlboro® Red 100 cigarettes were smoked and on the other visit Quest 3® cigarettes were smoked. The first smoking session was after abstinence from smoking for at least 10 h, verified by exhaled CO < 15 ppm. The second cigarette was smoked 25 minutes after the first to compare smoking in a non-abstinent state.

Each smoking session included fMRI and subjective rating data collection during a five-minute baseline period, a five-minute paced (1 puff every 21 seconds) air-puffing period with an unlit cigarette, a five minute period of paced cigarette smoking (1 puff every 21 seconds), and functional scanning for an additional 10-minute post-smoking period, for a total of 25 minutes. After the end of the post smoking period, participants had 5 minutes of additional scanning without subjective ratings questionnaires before the 25 minute smoking procedure outlined above was repeated with the same cigarette type as the previous scan (in a non-abstinent state).

In general, subjective ratings of smoking effects changed over the smoking period in a similar manner with both types of cigarettes. Increases in ratings of "High" and "Feel Drug Effects" occurred in every smoking session condition, regardless of the type of cigarette of whether it was smoked after abstaining or shortly after another cigarette. However, the magnitudes of the increases with Quest 3 ® VLN cigarettes were significantly smaller that with the Marlboro® Red 100 cigarettes.

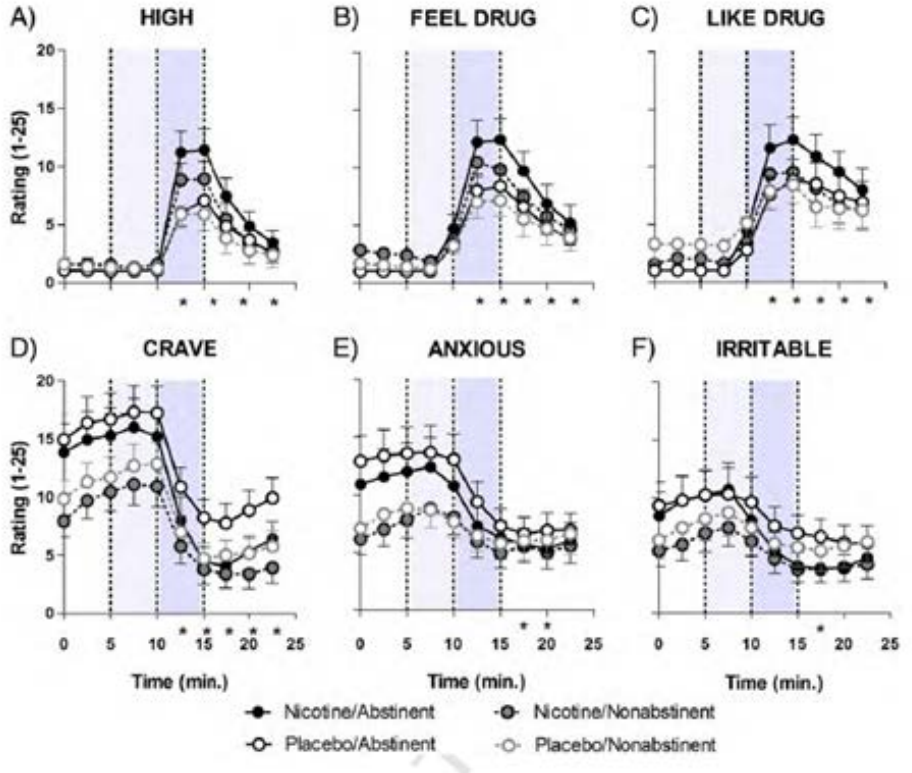


Fig. 2. Full time courses of smoking induced subjective ratings. All four conditions analyzed in the 2×2×9 ANOVA are shown. The air puffing period (minutes 5–10) and smoking period (minutes 10–15) are indicated by light gray and dark gray rectangles respectively. Time points showing a significant main effect of time are indicated on the X axis of each panel. (Significance from baseline time-point: *$p \leq 0.05$) Abstinence state exerted a significant main effect on all subjective ratings (Panels A–F). Cigarette type had a significant main effect on "High" (A), "Feel Drug" (B), "Like Drug" (C), and "Crave" (D). Cigarette type and abstinence state interacted significantly on ratings of "Like Drug" (C) with the condition in which a nicotine cigarette was smoked in an abstinent state producing the greatest changes in ratings.

From Lindsey et al. (2013) *Pharmacol Biochem Behav* 103(4):710-716

Ratings of "Crave" decreased significantly from baseline by the end of the cigarette smoking period under every smoking condition. During the time period in which cigarettes were actually being smoked, the difference in the degree of reduction between cigarette types was not significant ($p = 0.139$). The reduction of cravings was less persistent with Quest 3®. Cigarette type had no effect on smoking-induced changes in ratings of anxiety ($p = 0.442$) or irritability ($p = 0.175$).

1.5.2.13 A Comparison of the Effects of Smoking Cigarettes with Different Nicotine Content on Enhancement of Positive and Negative Reinforcement

A study by Perkins and Karelitz examined the effects of smoking Quest 3® VLN cigarettes or moderate nicotine content Quest 1® cigarettes on enhancement of positive reinforcers (money, music) or negative reinforcers (termination of noise).[14]

A total of 52 subjects, 25 dependent and 27 nondependent smokers, participated in four sessions in which reinforcement was assessed by responses on a simple operant computer task. In three sessions that followed overnight abstinence (confirmed by exhaled CO < 10 ppm), subjects smoked a Quest 3® VLN cigarette, smoked a Quest 1® cigarette, or did not smoke. In a fourth session non-abstinent subjects smoked their own usual brand cigarette *ad libitum*.

In each session subjects completed four 15 min trials that differed in the reward that was available as a reinforcer: (1) money, (2) preferred music, (3) termination for 30 sec of aversive noise, or (4) no reward. Prior to each of the four task trials in the session with Quest 3® or Quest 1® cigarettes, participants self-administered six puffs over 3 min, one puff every 30 sec, on the cigarette assigned for that session via the a portable smoking topography device, guided by computer-presented puffing instructions.

Mean total puff volume per trial was greater for the Quest 3 VLN cigarette compared to the Quest 1® cigarette, 339 ± 13 ml vs. 265 ± 12 ml, $p < 0.001$.

In the three sessions following overnight abstinence, overall responding for rewards was significantly influenced by the smoking condition ($p < 0.001$). Within-subjects pairwise comparisons showed higher reinforced responding per trial due to with the Quest 1® cigarette, 788± 42, which was 116±26 more than with the Quest 3® VLN cigarette ($p < 0.001$), and 111 ± 32 more than in the no smoking condition ($p < 0.001$).

When effects of smoking condition on responding for different rewards were analyzed, the effect was significant for music only ($p = 0.001$), and marginal for money ($p = 0.07$) and termination of aversive noise ($p=0.10$), and had no effect on responding in the control condition. There was no significant difference in responding after a Quest 3® VLN cigarette vs. the no smoking condition ($p = 0.33$).

1.5.2.14 Separate and Combined Effects of VLN Cigarettes and Nicotine Patch in Smokers with Schizophrenia and Control Smokers

A study by Tidey *et al*. examined the separate and combined effects of nicotine patch and Quest 3® VLN cigarettes on cigarette craving, withdrawal symptoms , and usual-brand smoking in in smokers with schizophrenia (n = 30) and control smokers (n = 26) who did not have immediate plans to quit.[15]

In Session 1, participants completed the questionnaires and then were asked to smoke one cigarette of their usual brand using a smoking topography measurement device to familiarize them with the device. In Session 2, participants smoked their usual-brand cigarettes using the smoking topography for 5 hours to measure their baseline smoking behavior. In Sessions 3-7, participants completed 5-hour controlled administration periods during which all cigarettes were smoked through the smoking topography device, and participants were cued to smoke according to the rate and timing of their smoking during Session 2. The order of the five smoking conditions: Quest 3® + 42 mg nicotine patch, Quest 3® + placebo patch, no cigarettes + NIC, no cigarettes + PLA, usual brand cigarettes + no patches, was counterbalanced across participants. After the 5-hour controlled administration periods, participants completed the subjective measures described below, followed by a 90-min period of *ad libitum* usual-brand smoking.

Schizophrenic smokers had higher smoke intake levels than control smokers during the 5-hour controlled administration periods, based on both CO boost (15.7 ± 13.8 ppm vs. 10.1 ± 11.5 ppm, $p = 0.05$) and total puff volume (7,145 ± 3,995 ml vs. 3,574 ± 1,641, $p < 0.01$). Schizophrenic smokers reported significantly higher scores than control smokers on the Minnesota Nicotine Withdrawal Scale MNWS ($p < 0 .001$) and for Habit Withdrawal ($p < 0.05$).

Smoking Quest 3® VLN cigarettes during the controlled administration periods reduced cigarette craving, nicotine withdrawal symptoms, and habit withdrawal symptoms. Averaged across diagnostic groups and nicotine replacement conditions, Questionnaire of Smoking Urges-scores with Quest 3® were 3.2 ± 1.8 vs. 5.0 ± 1.5 without VLN cigarettes ($p < 0.001$), MNWS scores were 17.3 ± 20.8 vs. 29.5 ± 24.1 ($p <0.001$), and Habit Withdrawal scores were 3.1 ± 1.6 vs. 3.7 ± 1.8 ($p < 0.01$).

The average total puff volume in the Quest 3® + nicotine patch condition was significantly lower than in the usual brand condition (4,969 ± 3,594 ml vs. 5,841 ± 4,004 ml, $p <0.01$), whereas the total puff volume from the VLN cigarette + placebo patch condition (5,268 ± 3,309 ml) was intermediate and did not significantly differ from either of those conditions.

Smoking Quest 3® during the controlled administration periods reduced usual-brand smoking during the subsequent ad libitum periods, based on both CO boost (Quest ®, 3.2 ± 0.2 ppm; No cigarette 9.8 ± 7.1 ppm) and total puff volume (Quest 3®, 1,803 ± 1,420 ml; No cigarette, 2,184 ± 1,498 ml). There was no effect of nicotine replacement on the amount of smoking in the subsequent usual brand *ad libitum* smoking period.

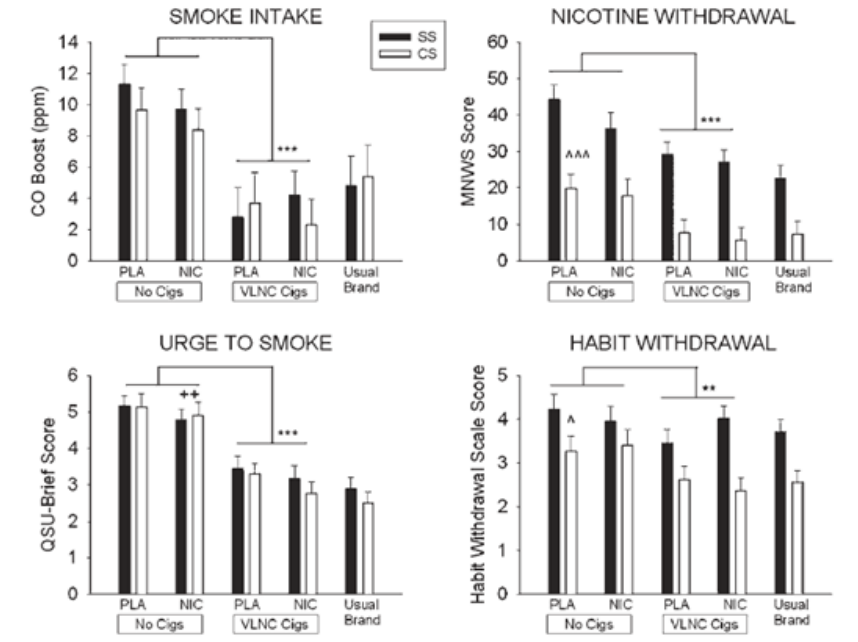


Figure 1. Effects of 5-h r exposure to 42 mg transdermal nicotine (NIC) or placebo patches (PLA) and very low nicotine content cigarettes (VLNC Cigs) or no cigarettes (No Cigs) on CO boost, urge to smoke, nicotine withdrawal symptoms and habit withdrawal symptoms in smokers with schizophrenia (SS; solid bars) and control smokers without psychiatric illness (CS; open bars). Bars represent M ± SEM. Significant main effects of sensorimotor replacement are indicated with asterisks (** $p < .01$; *** $p < .001$), significant main effects of nicotine replacement are indicated with plus signs (++ $p < .01$) , and significant main effects of group are indicated with carets (^ $p < 0.05$; ^^^ $p < 0.001$).

From Tidey et al. (2013) *Nicotine Tob Res* 2013; 15(1):121–129

VLN cigarettes with either nicotine patch or placebo patch received lower satisfaction and reward ratings than usual brand cigarettes, but were well accepted by both groups and did not affect psychiatric symptom levels in schizophrenic smokers.

2 SUMMARY INFORMATION

There were no active clinical trials during this reporting period.

3 NONCLINICAL STUDIES

3.1 Pharmacology

3.1.1 Ongoing Studies

There are currently no nonclinical pharmacology studies ongoing.

3.1.2 Completed Studies

There were no nonclinical pharmacology studies conducted with X-22 Smoking Cessation Product during this reporting period.

3.2 Pharmacokinetics/Absorption, Distribution, Metabolism, and Excretion

3.2.1 Ongoing Studies

There are currently no nonclinical pharmacokinetic/absorption, distribution, metabolism, and excretion (ADME) studies ongoing.

3.2.2 Completed Studies

There were no nonclinical pharmacokinetic/ADME studies conducted with X-22 Smoking Cessation Product during this reporting period.

3.3 Toxicology

3.3.1 Ongoing Studies

There are currently no toxicology studies ongoing.

3.3.2 Completed Studies

There were no toxicology studies conducted with X-22 Smoking Cessation Product during this reporting period.

4 CHEMISTRY, MANUFACTURING, AND CONTROLS

There is no new chemistry, manufacturing and controls information for this report.

5 GENERAL INVESTIGATIONAL PLAN

5.1 Broad Objectives for X-22 Smoking Cessation Product Development

The goal of the ongoing Phase II development program will be to evaluate the safety and efficacy profile of X-22 Smoking Cessation Product, the pharmacokinetics of nicotine in the X-22 cigarette, and to determine the optimum dosing regimen in order to design the pivotal Phase III clinical trials for the desired indication.

The ongoing study of the effects of Spectrum® research cigarettes containing tobacco blends with various nicotine concentrations[3] includes cigarettes with very low nicotine concentrations equivalent to those in cigarettes used in the 22nd Century Phase II trial (0.4 mg/g) and in the recently completed Queen Mary University of London trial[16] and University of Minnesota trial[1] (1.5 mg/g). Plans for further studies to evaluate the effects of nicotine concentration in the filler on nicotine intake, smoking behavior, and compliance will be finalized after the review of these studies.

Effects of nicotine concentration in the filler will be evaluated in Phase II studies. Single and multiple dose pharmacokinetics of nicotine in X-22 cigarette and relative bioavailability of nicotine from X-22 cigarettes compared to other sources of NRT will be finalized and the studies will be carried out prior to the commencement of Phase III studies.

Phase III studies will be conducted to further evaluate the safety and efficacy of X-22 cigarettes as an aid to smoking cessation.

5.2 Clinical Studies Planned for the Coming Year

22nd Century plans to initiate 2 clinical studies in the coming year. One will be a Phase II study of comparative effects of cigarettes with different filler nicotine content. The other will be effects of an open-label, randomized, crossover design study to evaluate the pharmacokinetics of nicotine obtained from X-22 Smoking Cessation Product in healthy smokers. Following completion of these studies, 22nd Century plans to start the first Phase III study of X-22 Smoking Cessation Product.

6 INVESTIGATOR BROCHURE

No changes were made to the Investigator's Brochure during this reporting period. The Investigator's brochure will be updated prior to initiation of the next clinical trial.

7 PHASE 1 PROTOCOL MODIFICATIONS

No amendments to a phase 1 study were made during this reporting period.

8 FOREIGN MARKETING DEVELOPMENTS

No foreign marketing applications for X-22 Smoking Cessation Product have been made or withdrawn nor has X-22 Smoking Cessation Product been suspended from marketing in any foreign country.

9 OUTSTANDING BUSINESS WITH RESPECT TO IND 103,589

There is no outstanding business related to IND 103,589.

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Published OnlineFirst April 19, 2013; DOI: 10.1158/1055-9965.EPI-12-1439

Cancer Epidemiology, Biomarkers & Prevention

Research Article

Reduced Nicotine Content Cigarettes and Nicotine Patch

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Abstract

Background: Reduced nicotine content (RNC) cigarettes have led to smoking fewer cigarettes, withdrawal relief, and facilitation of cessation. The aim of this study is to examine the effects RNC cigarettes with and without nicotine patch and patch alone on smoking behavior, toxicant exposure, withdrawal discomfort, and as an exploratory analysis, on long term abstinence.

Methods: This study involved a randomized, parallel arm design and six weeks of: (i) 0.05 0.09 mg nicotine yield cigarettes (*N* 79); (ii) 21 mg nicotine patch (*N* 80), or (iii) 0.05 0.09 nicotine yield cigarettes with 21 mg nicotine patch (*N* 76); all groups received six weeks of additional behavioral treatment with follow ups up to six months.

Results: Combination approach led to lower rates of smoking assigned cigarettes and hence lower carbon monoxide levels than RNC cigarettes alone. In addition, the combination approach was associated with less withdrawal severity when switching from usual brand to assigned product, and less smoking of usual brand cigarettes during treatment, but not after treatment compared with the other approaches.

Conclusion: Combining very low nicotine content cigarettes with nicotine patch may improve the acute effects resulting from switching to either of these products alone.

Impact: These findings may have implications for smoking cessation treatment or a policy measure to reduce nicotine content in cigarettes. *Cancer Epidemiol Biomarkers Prev; 22(6); 1015 24. ©2013 AACR.*

Introduction

The use of reduced nicotine content (RNC) cigarettes has been considered as a possible cessation tool and as a national policy measure (1 3). Unlike "light" cigarettes, the nicotine content in the RNC cigarette itself is substantially lower than conventional cigarettes. Reducing nicotine in cigarettes to levels that are nonaddictive would potentially lead to cessation from smoking (because cigarettes are no longer reinforcing) and if implemented as a policy, has the potential to have significant public health benefit.

To date, the scientific literature shows that switching to RNC cigarettes leads to a reduction in cigarette intake with minimal compensatory smoking behavior, no greater exposure to toxicants than their usual brand cigarettes, decrease in dependence and facilitation of abstinence among smokers not interested (4, 5), and interested in quitting smoking (3, 6). As an example of the effects of RNC cigarettes in facilitating cessation, smokers interested in quitting who were assigned to the 0.05 mg nicotine yield cigarettes achieved a biochemically verified 7-day point prevalence smoking cessation rate of 35.9% as compared with 13.5% and 20.0% among those smokers assigned to a higher nicotine yield cigarettes (0.3 mg nicotine yield) or to nicotine lozenge at 6 weeks posttreatment, respectively. Another large study found that smokers calling a quit line and assigned to RNC cigarettes plus usual care, which involved use of nicotine replacement therapy (NRT), were observed to have significantly higher abstinence rates than those who were provided usual care alone (3). To date, no study has examined the effects of combining RNC cigarettes plus nicotine patch on smoking behavior, resultant toxicant exposure, withdrawal symptoms and craving, dependence scores, and on abstinence rates compared with medicinal nicotine product alone and with RNC cigarettes alone. To address this gap, we conducted a study in which smokers were randomized to 6 weeks of 0.05 0.09 mg nicotine yield cigarettes, 21 mg nicotine patch, or a combination of both. We hypothesized that smoking behavior, toxicant exposure, withdrawal, and craving upon switching to the assigned product would be less for the combined intervention condition compared with the products alone. These hypotheses are based on the assumption that the RNC cigarettes would decrease craving associated with the sensory aspects of smoking and reduce the reinforcing value of cigarettes, whereas the patch would provide nicotine (not associated

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doi: 10.1158/1055 9965.EPI 12 1439

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Published OnlineFirst April 19, 2013; DOI: 10.1158/1055-9965.EPI-12-1439

with the act of smoking) for nicotine-related craving and withdrawal relief. Therefore, the combination therapy would lead to better treatment response than either product alone. In an exploratory analysis, we also hypothesized that abstinence rates would be highest with combined product treatment condition compared with the single product conditions.

Our goal was to examine the feasibility of using these cigarettes as a method to significantly reduce smoking behavior and the effects of adding the nicotine patch in augmenting beneficial effects from RNC cigarettes. If this combination approach proved more effective than the products alone, then RNC cigarettes can be considered an adjunct to existing NRTs. In addition, in the event of a national policy to reduce nicotine in all cigarettes, the results would suggest the importance of making NRTs easily accessible to smokers.

Materials and Methods

Subjects

Smokers between the ages of 18 and 70 interested in quitting smoking were recruited via advertisement from the Twin Cities and Duluth, Minnesota. In the advertisements, the study was described as testing a nicotine-free cigarette or new tobacco product as a way to become smoke free. To be eligible, smokers had to (i) have smoked 10 to 40 cigarettes daily for the past year (the range was instituted to reduce heterogeneity); (ii) be in good physical and psychiatric health; and (iii) have no contraindications for medicinal nicotine use. Smokers using other tobacco or nicotine products and smokers who were pregnant or nursing were excluded. The study was approved by our Institutional Review Board and in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services.

Study design

After a telephone screening to determine preliminary eligibility, an orientation session was held at which the study was further explained, written informed consent was obtained, and a more thorough screening for eligibility was conducted.

After a 2-week period during which baseline measurements were collected while subjects smoked their usual brand *ad libitum*, subjects were assigned to one of 3 conditions: (i) 0.05 0.09 mg nicotine yield cigarettes, that is very low nicotine content (VLNC) cigarettes, (ii) 21 mg nicotine patch, or (iii) combination of both. Subjects were initially assigned Quest 3 cigarettes (manufactured by Vector), a commercially available VLNC cigarette of $\leq$ 0.05 mg machine-determined nicotine yield, 0.7 to 0.9 mg nicotine and about 8 to 11 mg tar on per cigarette basis, and with reduced levels of tobacco-specific nitrosamines compared with conventional cigarettes [4-(methylnitrosamino)-I-(3-pyridyl)-1-butanone (NNK) = 0.05 and *N*-nitrosonornicotine (NNN) = 0.83 μg/g tobacco wet weight for Quest 3 vs. NNK = 0.68 and NNN = 2.8 μg/g tobacco wet weight for Marlboro "Light" (7)]. Quest 3 cigarettes no longer were available after randomizing 27% of our subjects, so we switched to Xodus (manufactured by 22nd Century Limited, LLC), which contained 0.09 mg machine-determined nicotine yield, 1.2 mg nicotine, about 10 mg tar and similarly low carcinogens (e.g., NNK = 0.05 μg/g and NNN = 0.72 μg/g tobacco wet weight, unpublished data). The average nicotine yield per each cigarette puff was about from 0.005 to 0.008 and 0.010 to 0.011 mg, for the 0.05 and 0.09 mg nicotine yield cigarettes, respectively. Subjects were instructed to use only assigned products for 6 weeks, after which time they were to discontinue all product use. Subjects were seen weekly during the 6-week product assignment period and an additional 6 weeks at weeks 7, 8, 10, and 12 for continued behavioral treatment.

At each visit, subjects assigned to either cigarette condition were provided a supply equivalent to 150% of their baseline smoking rate (to allow for compensatory smoking) and were told to smoke these VLNC cigarettes *ad libitum*, that is, as they would smoke their usual cigarettes. Subjects assigned to receive nicotine patch were informed to replace the old patch with the new patch each morning. Subjects maintained a daily smoking diary where they recorded any cigarettes smoked (either those assigned to them or their usual brand). They were not penalized for smoking unassigned cigarettes, but told that although we do not encourage them to smoke cigarettes other than those assigned, it is crucial to the study that they accurately report all cigarette use.

Brief standardized counseling was provided at each visit during the intervention phase of the study. During the first 6 weeks, subjects assigned to the cigarette conditions were counseled to consider the use of these products as a step toward quitting and discussed behavioral strategies to resist smoking other (non-VLNC) cigarettes. Subjects assigned to the nicotine patch only condition were provided treatment tools recommended by the U.S. Clinical Practice Guideline (8). During the second 6-week intervention phase, all subjects received counseling similar to that received by the subjects assigned to the nicotine patch condition. All 3 treatment groups received similar amounts of behavioral support.

Follow-up visits occurred at 16, 24, and 36 weeks. Subjects who completed the study were paid up to $330.

Outcome measures

Biomarkers of tobacco exposure measures included (i) urinary total nicotine equivalents (TNE), which is the sum of nicotine, cotinine, and 3′-hydroxycotinine and their glucuronides, altogether accounting for 73% to 96% of the nicotine dose (9, 10); (ii) urinary total cotinine, a metabolite of nicotine; (iii) alveolar carbon monoxide (CO) measured using the Bedfont Micro Smokerlyzer (Bedfont Scientific Limited); and (iv) urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL), metabolites of the tobacco-specific lung carcinogen NNK (11). All measures were assessed at baseline. In addition, CO was assessed at each clinic visit, cotinine at

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weeks 2, 6, and 12 of intervention and at follow-up visits, and biomarkers for other exposures at week 6 of intervention.

Subjective measures included: (i) a Tobacco Use Questionnaire asking about current tobacco use status (cigarettes and other tobacco products); (ii) a daily diary detailing the number of assigned products used and usual cigarettes smoked; (iii) the Minnesota Nicotine Withdrawal Scale, a widely used scale assessing withdrawal from cigarettes (12 14), nicotine gum (15, 16), and smokeless tobacco (15, 17), (iv) Fagerstrom test for nicotine dependence (FTND, 18), (v) Centers for Epidemiological Studies 20-item scale (CES-D) assessing current symptoms of depression (19), and (vi) perceived health risk, a ladder involving rating risk for addiction to a product on a scale ranging from 1 to 10 (6). All of these measures were assessed at baseline. Cigarette or product use was assessed daily, the Tobacco Use Questionnaire and Minnesota Nicotine Withdrawal Scale at each clinic visit, and perceived health risk at weeks 2 and 6.

Statistical analysis

Subjects' baseline characteristics including demographics and smoking history were described and compared among 3 intervention groups. Discrete variables were analyzed using Pearson χ^2 or Fisher exact tests. Contiuous variables were analyzed using either one-way ANOVA or Kruskal Wallis tests.

We conducted an intention-to-treat analysis. Biomarkers including TNE, cotinine, and NNAL were adjusted for creatinine and analyzed on the natural log scale to ensure normality; geometric means in original units are presented. Abstinence during the first 6-week treatment period was calculated as point prevalence abstinence from usual brand cigarettes for the past 7 days (by self-report). After this period, biochemically verified 7-day point prevalence abstinence and continuous abstinence rates during weeks 12, 16, 24, and 36 were calculated. Differences between treatment groups were evaluated using χ^2 tests. Dropouts were considered to have relapsed at the date of their last follow-up visit. Time to smoking relapse was calculated from the start of treatment to date of first relapse (defined as smoking of >4 usual brand cigarettes during treatment period or any tobacco use during follow-up). Those who remained abstinent throughout the study were censored at the time of last follow-up. Kaplan Meier methods were used to determine the median time to relapse and 95% confidence interval (CI) for each group.

All continuous outcomes with repeated measures from baseline through treatment were analyzed using mixed effects ANOVA models with fixed effects for site, treatment, visit, interaction between treatment and visit, and a random effect for subject. Least squares (LS) means and 95% CI are presented unless otherwise noted. The P values reported were adjusted for multiple comparisons as appropriate using a Bonferroni correction. $P < 0.05$ were considered statistically significant.

Results

Subjects

Figure 1 shows the consort diagram outlining the disposition of the subjects. Of the 316 who signed the informed consent form, 235 smokers ($n = 203$ from Minneapolis, Minnesota and $n = 32$ from Duluth, Minnesota) were randomly assigned to treatment (80 to nicotine patch, 79 to VLNC, and 76 to VLNC + nicotine patch).

No significant differences in demographics and smoking history, or biomarkers of exposure were observed across the treatment conditions at baseline (see Tables 1 and 2). Of those subjects who enrolled in the study, 173 subjects completed treatment ($N = 60$ in nicotine patch, $N = 55$ in VLNC, and $N = 58$ in VLNC + nicotine patch). The number of dropouts in each group at various stages throughout the study with reasons for dropouts is indicated in Fig. 1. All subjects were contacted for follow-up, accounting for the higher numbers during follow-up compared with the end of treatment. There were no significant differences in baseline characteristics between subjects who dropped out of the study after randomization and those who completed the entire study.

Product use during treatment

The number of assigned cigarettes smoked per day during the first 6-week treatment period is illustrated in Fig. 2A. There were significant treatment ($F_{(2,876)} = 130.88$, $P < 0.0001$), time ($F_{(5,876)} = 36.75$, $P < 0.0001$), and treatment by time ($F_{(10,876)} = 9.81$, $P < 0.0001$) effects observed. Significant differences in number of assigned cigarettes smoked were observed at each time point between the VLNC and VLNC + nicotine patch conditions ($P \leq 0.01$), with the exception of week 1 ($P = 0.063$); those assigned to the VLNC + nicotine patch smoked fewer cigarettes. At week 6, the mean ± SD number of VLNC cigarettes smoked in the VLNC condition was 16.2 ± 10.2 and in the VLNC + nicotine patch condition was 11.3 ± 7.6. Among subjects assigned to the 2 conditions with the nicotine patch, 100% of the nicotine patch group and 95.6% of the VLNC + nicotine patch group reported daily use of the patch at week 6.

Across treatment groups, significant differences in abstinence from nonstudy cigarettes (e.g., usual brand cigarettes) were observed during the 6-week product assignment period ($P = 0.0001$). In particular, those subjects in the VLNC + nicotine patch group were significantly more likely to be abstinent than either the nicotine patch or VLNC only groups ($P = 0.004$ and $P = 0.009$, respectively). Subjects were most likely to smoke usual brand cigarettes during the first week of treatment: 54.2% of those assigned to VLNC cigarettes, 63.8% of those assigned nicotine patch alone, and 41.5% assigned to the combined products group. After week 1, the percentage who reported using usual brand cigarettes ranged from

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25.4% to 38.2% (during weeks 2 through 6) in the VLNC cigarette group, 35.6% to 50.8% in the nicotine patch group, and 8.3% to 21.7% in the VLNC + nicotine patch group. Significant differences between groups were observed during each of these weeks (all $P < 0.02$). At the week 6 visit, 32.7% in the VLNC cigarettes group, 43.3%

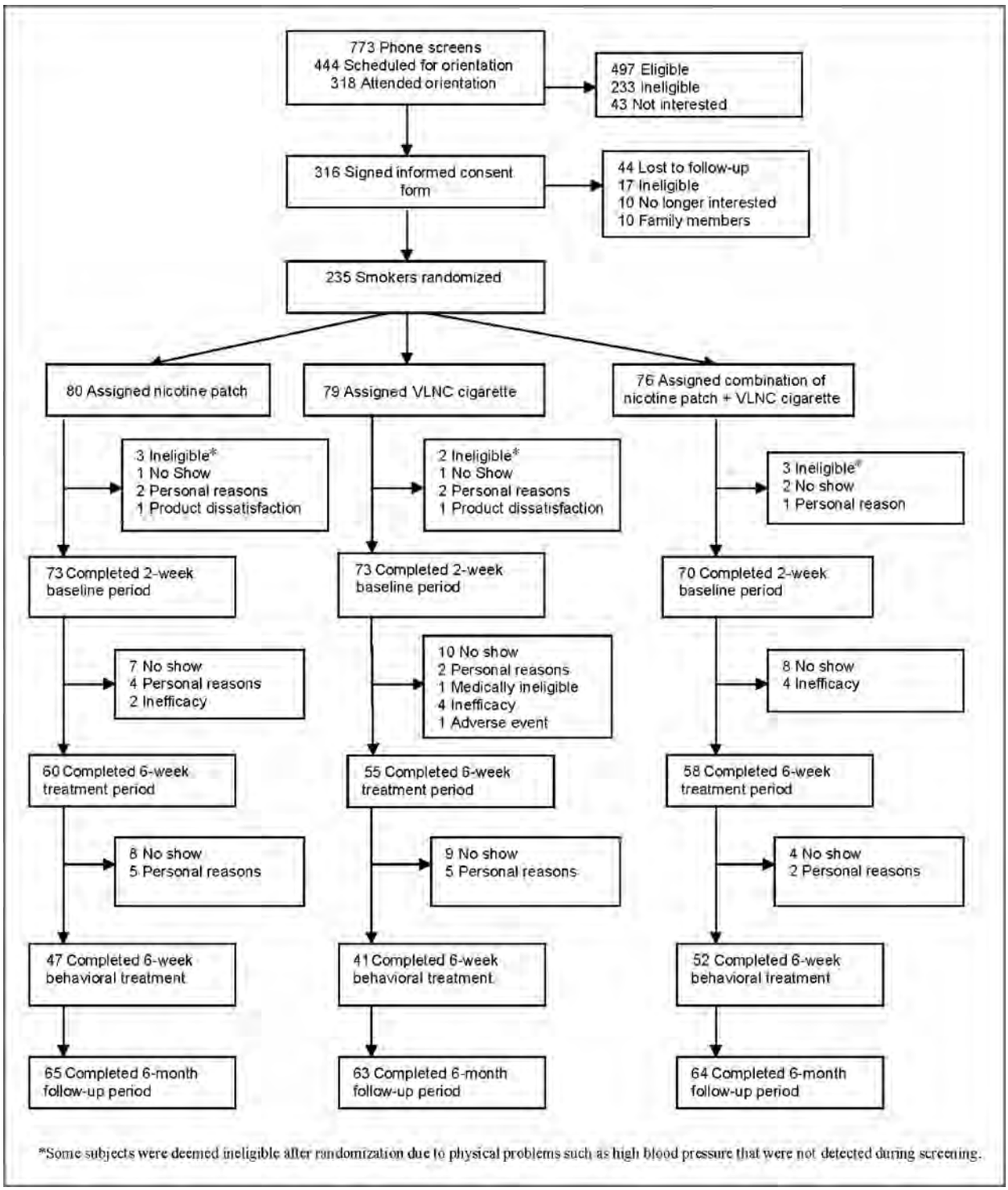


Figure 1. Flow of subjects through study.

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Table 1. Baseline demographics and smoking history of subjects by treatment group ($N = 235$)

	Overall	VLNC	NP	VLNC + NP
Age (y)	47.0 ± 11.7	46.5 ± 12.2	47.3 ± 11.0	47.0 ± 11.9
Female	57.9%	59.5%	57.5%	56.6%
Non-Hispanic Whites	82.0%	85.9%	84.8%	75.0%
Education				
8th grade or less	0.9%	2.6%	0.0%	0.0%
Some high school	2.6%	3.9%	1.3%	2.7%
High school graduate	22.8%	18.0%	26.3%	24.0%
Some college/2-year	56.7%	53.9%	58.8%	57.3%
College graduate	13.7%	18.0%	11.3%	12.0%
Graduate	3.4%	3.9%	2.5%	4.0%
Marital status				
Never married	26.8%	25.3%	28.9%	25.3%
Currently married	38.7%	41.8%	36.3%	41.8%
Currently not married	34.5%	32.9%	35.0%	32.9%
Cigarettes per day	18.9 ± 7.2	19.4 ± 6.2	19.5 ± 8.6	17.7 ± 6.3
Duration of having smoked regularly (y)	29.1 ± 12.0	29.2 ± 11.6	29.6 ± 11.7	28.4 ± 12.8
Age becoming a regular smoker (y)	17.9 ± 4.6	17.3 ± 3.6	17.7 ± 4.9	18.8 ± 5.0
Number of quit attempts				
0–2	23.5%	21.6%	22.4%	26.9%
3–5	39.6%	37.8%	40.8%	40.3%
6–10	22.6%	23.0%	22.4%	22.4%
11–20	11.1%	12.2%	11.8%	9.0%
20+	3.2%	5.4%	2.6%	1.5%
Motivation to quit (0–10 scale)	8.5 ± 1.4	8.5 ± 1.4	8.3 ± 1.5	8.6 ± 1.4
FTND	5.4 ± 1.9	5.6 ± 1.7	5.3 ± 2.1	5.1 ± 2.0
CES-D (0–60 scale)	10.6 ± 7.4	11.1 ± 7.7	10.9 ± 7.7	9.6 ± 6.7

NOTE: Because of missing values, the Ns were 217 for quit attempts, 224 for motivation to quit, 227 for FTND and 223 for CES-D. Otherwise, all other variables had data from 233 to 235 subjects.
Abbreviation: NP, nicotine patch.

in the nicotine patch group, and 13.8% in the combined products group reported using such products ($P = 0.002$). Among those reporting smoking nonstudy cigarettes during the treatment period, the mean number of self-reported usual brand cigarettes smoked ranged from 2.8 to at most 4.4 per week.

Effects of products on biomarkers of exposure during treatment

Exhaled CO during the treatment period is shown in Fig. 2B. Urinary TNE, total cotinine, and total NNAL adjusted for creatinine are presented in Table 2.

As illustrated in Fig. 2B, exhaled CO concentrations followed a similar pattern as seen for number of cigarettes smoked per day. There were significant treatment ($F_{(2,847)} = 53.99$, $P < 0.0001$), time ($F_{(5,847)} = 4.88$, $P = 0.0002$), and treatment by time ($F_{(10,847)} = 2.10$, $P = 0.022$) effects. All comparisons between CO levels for each treatment pair at each visit are significantly different from each other ($P \leq 0.05$).

Baseline TNE, total cotinine, and total NNAL levels were significantly higher than levels assessed at week 6 for each of the products (all $P < 0.007$). Compared with subjects assigned to the VLNC condition, subjects assigned to the nicotine patch and VLNC + nicotine patch conditions had significantly higher TNE levels ($P = 0.0005$ and $P = 0.0001$, respectively) and total cotinine levels ($P = 0.021$ and $P = 0.002$, respectively) at week 6. Subjects assigned to nicotine patch had significantly lower total NNAL than VLNC users at week 6 ($P = 0.024$), but no significant differences were observed between subjects assigned to VLNC + nicotine patch versus VLNC cigarettes ($P = 0.276$).

Effects of products on subjective responses during treatment

Dependence. Perceived heath risk score for addiction during treatment is illustrated in Fig. 3A. Significant decreases were observed across all treatments compared with baseline ($P < 0.0001$); no differences were observed across treatments. Nicotine craving and withdrawal symptoms during the 6-week product assignment period and 1 week after this period are illustrated in Fig. 3B and C. Upon cessation of usual brand cigarettes and switching to the products (week 1 compared with baseline), there was a significant decrease in craving ($P = 0.0002$) and increase in

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Table 2. Geometric means of biomarkers at baseline and week 6 of treatment period by treatment groups

	Baseline		Week 6	
Biomarkers	***N***	**Geometric mean (95% CI)**	***N***	**Geometric mean (95% CI)**
Total TNE[a]				
VLNC Cigarette	54	55.70 (48.42, 64.07)	54	6.89 (4.26, 11.02)
Patch	59	49.90 (43.82, 57.40)	58	23.10 (14.59, 36.60)
VLNC Cigarette + Patch	58	53.52 (46.99, 61.56)	58	27.39 (17.29, 43.38)
Total cotinine[a]				
VLNC cigarette	54	17.12 (14.44, 20.09)	54	2.03 (1.20, 3.45)
Patch	59	16.78 (14.30, 19.69)	59	5.50 (3.31, 9.13)
VLNC cigarette + patch	58	17.99 (15.33, 21.12)	58	7.65 (4.59, 12.76)
Total NNAL[b]				
VLNC cigarette	53	1.20 (1.00, 1.43)	52	0.40 (0.29, 0.55)
Patch	59	1.29 (1.09, 1.53)	59	0.25 (0.18, 0.34)
VLNC cigarette + patch	57	1.09 (0.92, 1.30)	55	0.26 (0.19, 0.36)

NOTE: Values are for all subjects from whom data were collected at the visit in question.
[a]nmol/mg creatinine.
[b]pmol/mg creatinine.

withdrawal symptoms ($P < 0.0001$) across all 3 treatment groups. For craving, no significant differences were observed between treatments. Increase in nicotine withdrawal scores upon cessation of usual brand cigarettes was significant by treatment group ($P = 0.008$); those assigned to VLNC + nicotine patch had significantly lower withdrawal symptoms than nicotine patch alone ($P = 0.008$), but only borderline significantly lower than VLNC alone ($P = 0.092$). No differences were observed between nicotine patch versus VLNC alone. Upon cessation of the product (week 7 compared with week 6), a significant increase in craving was observed ($P < 0.0001$), but no differences among treatments. For withdrawal symptoms, a significant change was observed ($P < 0.0001$), with withdrawal symptoms lower in week 7 compared with week 6 for those assigned to the nicotine patch and slightly higher in week 7 for those assigned to the VLNC or VLNC + nicotine patch groups. These differences were not quite statistically significant among treatments ($P = 0.110$).

Abstinence

After completion of the assigned product treatment period, biochemically verified (CO < 6 ppm to rule out cigarette use) point prevalence rates of abstinence from cigarettes at each of the follow-up visits and continuous abstinence rates (at weeks 12, 24, and 36) showed no significant differences across treatment groups (Table 3). Similar results were observed for abstinence from all nicotine-containing products (CO < 6 ppm, cotinine < 35 ng/mL). If subjects who never received the product were excluded from the analysis, the rates of point prevalence abstinence at week 36 across the conditions would range from 19.2% to 21.4%. The median time to relapse (95% CI) since treatment onset was 7.1 (6.7 7.7) weeks for VLNC + nicotine patch, 2.6 (1.7 5.9) weeks for VLNC, and 2.1 (1.6 3.9) weeks for nicotine patch.

Discussion

The combination of VLNC + nicotine patch led to significantly lower rate of smoking assigned cigarettes and hence lower CO levels compared with VLNC

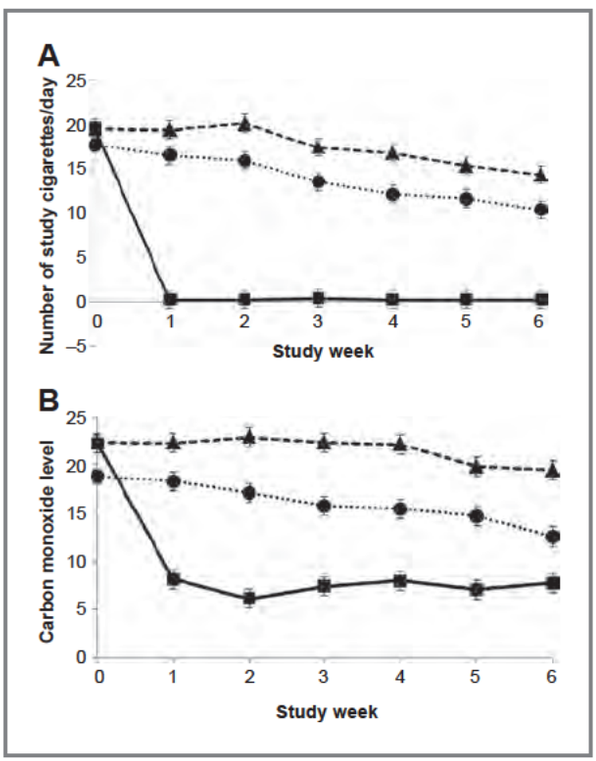


Figure 2. LS mean (±SE) of number of study cigarettes smoked per day (A) and exhaled carbon monoxide (B). Triangle represents VLNC cigarette alone; square represents nicotine patch alone; circle represents VLNC + NP.

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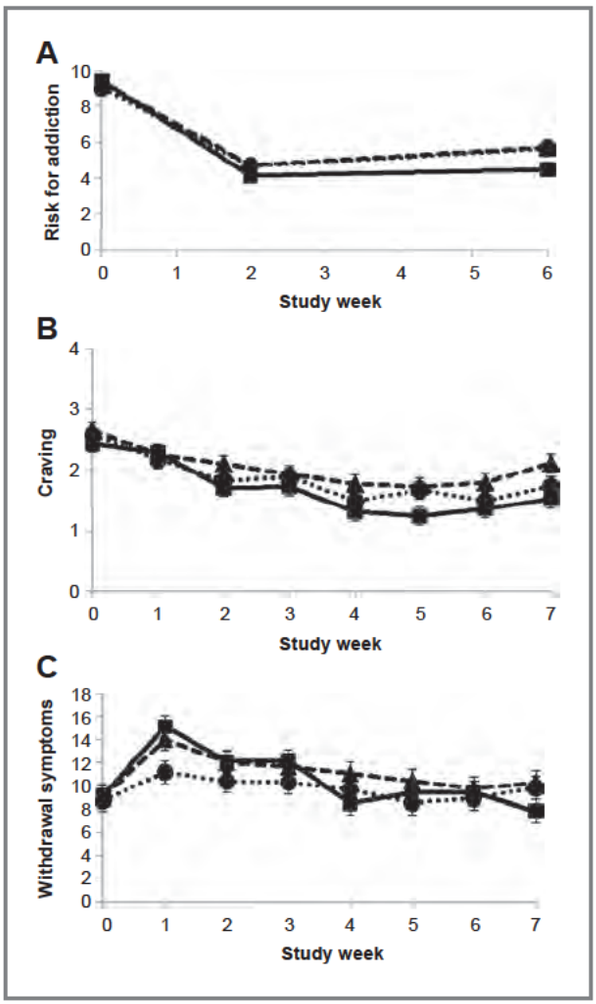


Figure 3. LS mean (±SE) of perceived health risk addiction score at baseline, week 2 and week 6 (A). LS mean (±SE) of craving and withdrawal symptoms (B and C) at baseline through week 7. Triangle represents VLNC cigarette alone; square represents nicotine patch alone; circle represents VLNC + NP.

cigarettes alone. As expected, both nicotine patch and VLNC + nicotine patch conditions resulted in higher levels of cotinine and TNE than VLNC alone condition. All treatment conditions showed a significant reduction in biomarkers of exposure compared with baseline. The combination condition also resulted in lower severity of withdrawal when switching from usual brand cigarettes to the assigned products (although only nearly significant different from VLNC alone), with no difference between nicotine patch and VLNC only conditions. Cessation from product use led to an increase in craving with no differences across groups and a change in withdrawal symptoms. Although not significantly different across groups, withdrawal symptom severity decreased with patch but increased in the conditions that used VLNC. Most importantly, the amount of usual brand smoking was lowest in the combination condition during the product assignment period. Thus, in general, the combination approach performed significantly better on many outcome variables than the conditions alone. However, exploratory analysis showed that after the product assignment, higher rates of usual brand cigarette abstinence in the combination condition were not sustained and no differences were observed across conditions.

The results from the VLNC cigarettes observed in this study are concordant with findings from a prior study that we conducted in which a VLNC cigarette (Quest 3) was compared with a higher reduced nicotine content cigarette and with the nicotine lozenge (6). The VLNC cigarettes led to reduced rates of smoking and reduced levels of CO, cotinine, and total NNAL levels compared with baseline and no greater withdrawal symptoms or differences in treatment outcome compared with nicotine lozenge alone.

Five other studies have examined the use of the nicotine patch in combination with the VLNC cigarettes. In a 10-day laboratory study conducted by Donny and Jones (20), subjects ($N = 68$) were randomly assigned one of 4 conditions: (i) placebo patch plus nicotine-containing cigarettes (Quest 1, 0.6 mg nicotine yield, 0/NC); (ii) placebo patch plus VLNC cigarettes (Quest 3, 0.05 mg nicotine yield, 0/VLNC); (iii), 7 mg nicotine patch plus VLNC cigarettes (7/VLNC), and (iv) 21 mg nicotine patch plus VLNC (21/VLNC). Consistent with our findings, subjects assigned to the 7 or 21/VLNC compared with 0/VLNC showed a greater decrease in the number of VLNC cigarettes smoked. These subjects also showed a greater decrease in total volume of VLNC cigarette smoke inhaled. Similarly, there was a trend toward participants in the 21/VLNC to show a greater decrease in CO relative to baseline and significantly less increase in CO boost after smoking the VLNC cigarette than participants in the 0/VLNC. Finally, greater withdrawal symptom relief was observed in the 7 or 21/VLNC compared with the 0/VLNC during a required abstinence period when subjects used their assigned products in a laboratory setting.

In another small, pilot treatment study ($N = 16$ 17 in each condition), 2 weeks before quit date, smokers were assigned to nicotine or placebo patch in each of 3 cigarette conditions containing different levels of nicotine (21). Relevant to our study, during 2 weeks before the quitting date, subjects assigned to VLNC cigarettes (0.08 mg nicotine) reported smoking 3 usual brand cigarettes in nicotine patch condition as opposed to 46 usual brand cigarettes in the placebo condition. In addition, in the VLNC condition, nicotine patch compared with placebo patch treatment was associated with a lower number of total cigarettes smoked per day but no differences were observed in CO levels, or effects on craving or withdrawal symptoms.

Walker and colleagues in 2012 (3) conducted a large randomized controlled trial to determine the effects of VLNC cigarettes (Quest 3) plus usual Quitline care (NRT and behavioral support) versus Quitline care alone on smoking abstinence. Smokers randomized to VLNC

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Table 3. Continuous (since week 6) and point-prevalence (past 1 week) posttreatment abstinence rates

CO- and cotinine-verified continuous abstinence

	Treatments						
	VLNC cigarette (*n* = 79)		Nicotine patch (*n* = 80)		VLNC + NP (*n* = 76)		
Week	# abstinent	%	# abstinent	%	# abstinent	%	*P*
12	11	13.9	11	13.8	8	10.5	0.776
24	9	11.4	10	12.5	6	7.9	0.625
36	8	10.1	8	10.0	6	7.9	0.867

CO-verified point prevalence abstinence

	Treatments						
	VLNC cigarette (*n* = 79)		Nicotine patch (*n* = 80)		Combination (*n* = 76)		
Week	# abstinent	%	# abstinent	%	# abstinent	%	*P*
12	21	26.6	24	30.0	22	29.0	0.888
16	21	26.6	20	25.0	23	30.3	0.752
24	18	22.8	17	21.3	16	21.1	0.959
36	15	19.0	19	23.8	16	21.1	0.763

CO- and cotinine-verified point prevalence abstinence

	Treatments						
	VLNC cigarette (*n* = 79)		Nicotine patch (*n* = 80)		Combination (*n* = 76)		
Week	# abstinent	%	# abstinent	%	# abstinent	%	*P*
12	19	24.1	19	23.8	18	23.7	0.998
24	15	19.0	16	20.0	13	17.1	0.896
36	14	17.7	15	18.8	15	19.7	0.950

NP, nicotine patch.

cigarettes were instructed to use these cigarettes whenever they had an urge to smoke for up to 6 weeks after their quit date. The results showed that more subjects withdrew in the usual care group compared with the group assigned the VLNC cigarettes (32 vs. 11 at 6 months). Furthermore, the group assigned the VLNC cigarettes had higher 7-day point prevalence abstinence rate at the 6 month follow-up compared with usual care (33% vs. 28%, Relative Risk (RR) = 1.18, 95% CI 1.01 1.39) and higher continuous abstinence rates (23% vs. 15%, RR = 1.50, 95% CI 1.20 1.87). Median time to relapse in the group assigned VLNC cigarettes was 2 months compared with 2 weeks in the usual care. Thus, unlike the results from the current study, the combination approach seemed to improve long-term cessation rate.

Two other studies were conducted that did not directly examine the effects of adding nicotine patch to VLNC cigarettes, but showed the principle that providing nicotine through the use of nicotine patch but dissociating the direct delivery of nicotine with cigarette smoking through the use of VLNC cigarettes may ease craving (22) or facilitate cessation (23) compared with continued use of higher level nicotine containing cigarettes before cessation (24).

The results from our studies and other studies may have implications both for treatment and for a potential national policy measure. For treatment, studies support the notion that targeting both the sensory aspects of smoking and nicotine addiction through the slow delivery nicotine (thereby dissociating smoking with the delivery of nicotine) provides greater withdrawal relief and may minimize use of usual brand cigarettes while on treatment or, based on the findings of other studies, at follow-up.

A national policy measure to reduce the levels of nicotine to nonaddicting levels will undoubtedly require access to different pharmacotherapies for tobacco cessation. The availability of the pharmacotherapies may not only reduce the discomfort associated with the reduced nicotine content cigarettes but may lead to substantial reductions in cigarette smoking and possibly to eventual cessation of all products.

There are several limitations to this study. First, no placebo patch was provided so it is unclear as to whether smoking reduction was due to subject concern about smoking and using the patch at the same time. On the other hand, this study represents more naturalistic comparisons of the effects combining both products.

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Published OnlineFirst April 19, 2013; DOI: 10.1158/1055-9965.EPI-12-1439

Furthermore, Donny and Jones (20) incorporated placebo patch in their study and observed similar results. Second, the short duration on product may have led to insignificant differences in treatment outcome during follow-up. In addition, for a clinical trial, the sample size was quite small. Third, VLNC cigarettes were switched in the middle of the study because the manufacturer had stopped making the initial cigarettes that were used. Therefore, the level of nicotine was increased. However, prior studies showed that significant reduction in cigarette smoking occur when cigarettes reach less than 0.1 mg nicotine yield (5, 25). In addition, when analyzing only those who received the Xodus product, the results were similar. Finally, we were unable to verify self-reported abstinence from usual cigarettes during product assignment. However, the patterns of biomarkers of exposures across the groups did not indicate that one group was more likely to report inaccurate data than another group.

In summary, the results from this study suggest that combining nicotine replacements with VLNC cigarettes may improve any acute effects resulting from switching to VLNC compared with VLNC alone and lead to a greater reduction in withdrawal discomfort or use of usual brand cigarette during treatment compared with the nicotine patch alone.

Disclosure of Potential Conflicts of Interest

D.K. Hatsukami had a commercial research grant from Nabi Biopharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: D.K. Hatsukami
Development of methodology: D.K. Hatsukami, S.S. Hecht, A.M. Joseph, S.S. Allen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.K. Hatsukami, L.A. Hertsgaard, S.E. Murphy, S.G. Carmella, M. al'Absi
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D.K. Hatsukami, R.I. Vogel, S.E. Murphy, S.S. Hecht, A.M. Joseph, S.S. Allen
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Grant Support

This work was supported by NIH, R01 DA025598 and U54DA031659. Sources of funding for all authors are NIH and their respective departments. D. Hatsukami had received funding from Nabi Biopharmaceuticals to conduct a trial on the nicotine vaccine. Clinical Trial Registration: clinicaltrials.gov identifier: NCT101050569.

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Received December 21, 2012; revised February 25, 2013; accepted March 27, 2013; published OnlineFirst April 19, 2013.

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ORIGINAL INVESTIGATION

Dose–Response Effects of Spectrum Research Cigarettes

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Received July 21, 2012; accepted October 10, 2012

ABSTRACT

Introduction: Experimental cigarettes are needed to conduct studies examining the effects of varying doses of nicotine content on smoking behavior. The National Institute on Drug Abuse contracted with Research Triangle Institute to make such cigarettes available to researchers. The goal of this study was to determine whether cigarettes that vary in nicotine content produce an expected dose–response effect.

Method: Two studies were conducted. The first study recruited subjects from 3 sites and consisted of a single, within-subject laboratory session. Subjects first smoked 4 puffs on their usual-brand cigarette and then in double-blind, random-order, smoked 4 puffs on each experimental cigarette that contained either low nicotine (LN, 0.4 mg/g), intermediate nicotine (IN, 5.7–5.8 mg/g), or high nicotine (HN, 11.4–12.8 mg/g). Each puffing bout was separated by a 30-min interval. Subjects completed questionnaires and were assessed for vital signs after each cigarette. The second study involved 1 site and used a between-subject design in which subjects were assigned to 1 of the 3 experimental cigarettes for 1 week. Subjective responses and biomarkers of exposure were assessed.

Results: In the first study, significant dose–response effects were observed, particularly between the LN and HN cigarettes. The second study showed decreases in cigarette smoking and exposure biomarkers predominantly in the LN group, with no changes in the HN cigarette group.

Conclusions: These results are similar to those observed in prior literature, confirming that these experimental cigarettes can be used safely and with the expected pharmacological effects.

INTRODUCTION

Smoking remains a leading cause of preventable disease and premature death worldwide. Approximately one in five death is associated with cigarette smoking, and roughly half of all daily smokers will die prematurely from tobacco-related illness (Doll, Peto, Boreham, & Sutherland, 2004; Peto, Lopez, Boreham, Thun, & Heath, 1992). Nicotine is the primary addictive agent in tobacco products (U.S. Department of Health and Human Services, 1988, 2010), yet a comprehensive body of scientific literature examining the effects of nicotine reduction in cigarettes or other tobacco products does not exist (Hatsukami, Perkins et al., 2010). The availability of cigarettes with varying levels of nicotine but otherwise similar characteristics provides the opportunity to improve understanding of how nicotine and other aspects of smoking contribute to the addictive properties of cigarettes.

The availability of cigarettes varying in nicotine content is also important to scientifically determine if reducing nicotine content in cigarettes may be a viable national policy strategy. Reducing the nicotine in cigarettes to the point that they are rendered nonaddictive has the potential to significantly reduce tobacco-related mortality and morbidity by decreasing the initiation of smoking and promoting cessation (Benowitz & Henningfield, 1994; Gray et al., 2005; Zeller, Hatsukami, & Strategic Dialogue on Tobacco Harm Reduction Group, 2009). The Family Smoking Prevention and Tobacco Control Act (FSPTCA) enables the Food and Drug Administration to establish tobacco product standards, including placing limits on the allowable nicotine content of cigarettes without reducing levels to zero. Similarly, Article 9 in the Framework Convention on Tobacco Control describes the regulation of content and emissions of tobacco products.

Currently, no reduced nicotine cigarettes are available to researchers that would allow examining the effects of varying doses of nicotine on smoking behavior. To meet this need, the National Institute on Drug Abuse (NIDA) contracted with Research Triangle Institute (RTI) to assist in the development of cigarettes varying in nicotine content. At least 9 million

doi:10.1093/ntr/nts247
Advance Access publication November 22, 2012
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cigarettes will be made available to the research community. After early batches were produced, the subjective effects, cardiovascular effects, and levels of nicotine and smoke exposure were determined in two pilot studies. In the first study, smokers were asked to take four puffs on their usual-brand cigarette and experimental cigarettes with three different nicotine content levels. In the second study, subjects were randomly assigned to one of three different nicotine content cigarettes for 1 week.

STUDY 1

Methods

Subjects

Subjects were recruited at three different sites, the University of Minnesota, University of Pittsburgh and the NIDA Intramural Research Program. Subjects who had previously enrolled in a smoking study or who responded to an advertisement for a smoking study were screened over the telephone to determine if they met the following eligibility criteria: 18–64 years of age, smoke at least 10 cigarettes/day with a CO > 10 parts per million (ppm) at screening, inhale when they smoked, use other forms of tobacco less than 10 days in the last 30 days, no plans to reduce or quit smoking, no use of nicotine replacement therapy, bupropion, or varenicline in the past 3 months, in good mental and physical health, not be pregnant, and not taking certain prescription medications or illicit drugs more than twice per week for the last month.

Cigarettes

Both menthol and nonmenthol cigarettes were manufactured by 22nd century (named Spectrum) under a NIDA contract with RTI. The nicotine content of the menthol and nonmenthol cigarettes as assessed by RTI were about 0.4 mg/g, 5.7–5.8 mg/g, and 11.4–12.8, mg/g, respectively (variation between menthol and nonmenthol cigarettes). The nicotine yield of these cigarettes (menthol and nonmenthol) as measured by the International Organization for Standardization method were <0.04 mg nicotine (low nicotine, LN), 0.3 mg nicotine (intermediate nicotine, IN), and 0.6 mg nicotine (high nicotine, HN) per cigarette. Tar yields, determined by Arista, were approximately 8.1–8.4, 8.6, and 9.6–9.8 mg, respectively. Menthol content, determined by RTI, were 1.23, 1.23, and 1.08 mg/g, respectively, for menthol cigarettes (yields were 0.47, 0.35, and 0.33 mg/cigarette, respectively) and nondetectable for nonmenthol cigarettes. Cigarettes did not vary in ventilation; instead, nicotine yields were achieved by blending tobacco with different nicotine content.

Study Design

Subjects attended one laboratory session during which informed consent was obtained and eligibility further assessed. Subjects first smoked their usual brand of cigarettes and then were asked to smoke in double-blind, random-order and sequential manner each of the three experimental cigarettes; each cigarette type was separated by 30-min intervals. Menthol and nonmenthol cigarettes smokers were assigned their respective preference. Subjects took four puffs (1 bout) on each cigarette type at 30-s interpuff intervals. Heart rate and blood pressure were measured immediately after each puffing bout. Alveolar carbon monoxide (CO) level was measured 2 min after each bout. Subjects then completed subjective rating scales, including the modified Cigarette Evaluation Questionnaire (mCEQ; Westman, Levin, & Rose, 1992) scored on an visual analog 0–100 scale (not at all to extremely) instead of a 1–7 scale, the Multiple Choice Procedure (MCP; Jacobs & Bickel, 1999), and the Perceived Health Risk (PHR) scale (measures perceived risk of experiencing a specific disease as a result of smoking experimental cigarettes; rated from very low risk of disease to very high risk of disease). Additional measures included assessment of characteristics of the tobacco product (e.g., flavorful, strength, harshness, amount of nicotine, like and dislike of cigarettes rated on a 0–100 scale, not at all to extremely). After the last cigarette, subjects were asked to rank the cigarettes in terms of their overall preference, including their usual brand.

Statistical Methods

Demographic and smoking history data were summarized by study site and menthol status. The mCEQ was scored as 5 subscales: Satisfaction, Psychological Reward, Aversion, Enjoyment of Respiratory Tract Sensations, and Craving Reduction (Cappelleri et al., 2007). All continuous outcomes were analyzed using a mixed effects analysis of variance model with fixed effects for baseline response (relating to their usual brand), experimental cigarette nicotine content, experimental cigarette smoking ordering, gender, study site, menthol status, interactions between nicotine content and menthol status and gender, and a random effect for subject. Rank data were analyzed using a proportional odds model with a cumulative logit link, with fixed effects for experimental cigarette nicotine content, experimental cigarette smoking ordering, gender, study site, menthol status, interactions between nicotine content and menthol status and gender, and a random effect for subject to account for the repeated nature of the data. Least-squares (LS) means ± standard errors (*SE*) were reported for each nicotine level unless otherwise noted and *p* values were adjusted for multiple comparisons using a Bonferroni correction. All significance levels were set at .05.

Results

Demographic and smoking history information of subjects recruited at the University of Minnesota (n = 20), University of Pittsburgh (n = 19), and NIDA (n = 12) are the following: mean age 39.6 years (SD = 12.6); 39.2% female; 58.8% White, 37.3% Black, and 3.9% other; 47.1% menthol smokers; mean cigarettes/day 18.6 (SD = 7.3); mean years daily smoking 19.9 years (SD = 11.9). No significant differences were observed across the sites. Information from two subjects at the University of Pittsburgh was lost for their first cigarette due to a computer malfunction. All others have complete data.

Modified Cigarette Evaluation Questionnaire

Table 1 shows the results for the mCEQ. For the subscales related to Satisfaction, Psychological Reward, Enjoyment of Respiratory Tract Sensation, and Craving Reduction, the smokers scored the HN and/or IN cigarettes significantly higher than the LN cigarettes. IN and HN cigarette scores were not significantly different on these scales. Nonmenthol compared with menthol smokers found their experimental cigarettes significantly more satisfying (59.1 ± 3.5 vs. 42.4 ± 3.8; F = 11.66, p = .001), more psychologically rewarding (43.5 ± 3.1 vs. 35.1 ± 2.9; F = 5.39, p = .022), more pleasing to the respiratory tract (52.3 ± 3.6 vs. 38.9 ± 3.9; F = 7.27, p = .008) and greater craving reduction (58.2 ± 3.8 vs. 45.9 ± 4.2; F = 5.33,

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Table 1. Study 1: Subscales of the Modified Cigarette Evaluation Questionnaire (mCEQ) by Nicotine Level

Measurement[a] (0–100 Scale)	Usual Brand Mean (*SE*)	Lower LS Means (SE)[b]	Intermediate LS Mean (*SE*)[b]	Higher LS Mean (*SE*)[b]	Lower vs. Intermediate	Lower vs. Higher	Intermediate vs. Higher
Satisfaction	83.6 (1.4)	36.8 (4.1)	54.1 (4.1)	61.4 (4.1)	$\|t\| = 3.26$*	$\|t\| = 4.65$***	$\|t\| = 1.38$
Psychological Reward	53.7 (2.2)	33.5 (3.1)	40.9 (3.1)	43.6 (3.1)	$\|t\| = 1.85$	$\|t\| = 2.53^{+}$	$\|t\| = 0.68$
Aversion	13.0 (1.7)	16.7 (2.8)	14.1 (2.8)	18.2 (2.8)	$\|t\| = 0.73$	$\|t\| = 0.40$	$\|t\| = 1.14$
Enjoyment of Sensation	73.1 (2.4)	32.7 (4.2)	49.3 (4.2)	54.8 (4.2)	$\|t\| = 2.70$*	$\|t\| = 4.09$**	$\|t\| = 1.03$
Craving Reduction	62.8 (3.3)	42.8 (4.5)	54.9 (4.5)	58.6 (4.5)	$\|t\| = 2.07$	$\|t\| = 2.71^{+}$	$\|t\| = 0.64$

Notes. LS = least square; SE = standard error.
[a]Subscale scores were averaged across the items.
[b]Adjusted for baseline response (usual brand), gender, menthol use, study site, cigarette order, interactions between nicotine level and menthol and gender, and repeated measures across subjects.
$^{+}p \le .05$. *$p \le .01$. **$p \le .001$. ***$p \le .0001$.

$p = .023$), but no significant interaction effect was observed between nicotine content and menthol status (data not shown). Women reported greater craving reduction than men (58.4±4.6 vs. 45.9±3.8; $F = 5.91$, $p = .016$). No significant gender or nicotine content by gender interactions were observed across the subscales other than craving reduction. For the Aversion subscale, participants did not score the experimental cigarettes differently and no menthol status or nicotine content by menthol status interaction effect was observed.

Other Subjective Responses to Spectrum Cigarettes

Significant differences were observed between LN and HN cigarettes for items measuring cigarette strength (30.1±4.9 vs. 46.0±4.8; $|t| = 2.54$, $p = .037$), flavorfulness (32.5±4.3 vs. 52.5±4.3; $|t| = 3.61$, $p = .001$), estimate of amount of nicotine in cigarettes (2.7±0.16 vs. 3.4±0.16; $|t| = 3.68$, $p = .001$), and liking (35.0±4.4 vs. 58.1±4.3; $|t| = 4.13$, $p = .0002$) and disliking (57.6±4.9 vs. 31.2±4.8; $|t| = 4.22$, $p = .0001$) of the experimental cigarette, with higher values assigned to the HN cigarettes except for disliking. Similar significant differences were observed between LN and IN cigarettes for liking (35.0±4.4 vs. 54.2±4.3; $|t| = 3.42$, $p = .003$) and disliking (57.6±4.9 vs. 39.5±4.8; $|t| = 2.89$, $p = .014$). Nonmenthol smokers compared with menthol smokers reported their cigarettes to be significantly more flavorful (52.4±3.7 vs. 33.5±4.0; $F = 13.64$, $p = .0003$), liked their cigarettes more (56.1±3.7 vs. 42.1±4.0; $F = 7.44$, $p = .007$), and disliked their cigarette less (33.8±4.1 vs. 51.6±4.5; $F = 9.63$, $p = .002$). No significant differences were observed for harshness of cigarette and on any of the measures between the IN and HN cigarettes. No differences between men and women were observed.

Both HN and IN cigarettes were associated with higher monetary value than LN cigarettes when subjects were asked the price at which they would switch to money over a pack of cigarettes ($4.88±0.40, $4.90±0.40, $3.44±0.41; $|t| = 2.75$, $p = .020$ for HN vs. LN and $|t| = 2.78$, $p = .019$ for IN vs. LN) and similarly for nonmenthol compared with menthol smokers ($5.15±0.34 vs. $3.66±0.38; $F = 9.58$, $p = .002$). The nicotine content by gender interaction was significant ($F = 3.147$, $p = .047$). Stratification by gender indicated that the significant differences in price by nicotine content shown above were evident among women ($5.01±0.66, $5.68±0.66, $2.88±0.66; $|t| = 2.38$, $p = .065$ for HN vs. LN and $|t| = 3.13$, $p = .009$ for IN vs. LN) but not men ($4.88±0.46, $4.15±0.47, $4.00±0.47; $|t| = 1.42$, $p = .484$ for HN vs. LN and $|t| = 0.24$, $p = 1.00$ for IN vs. LN).

When ranking the cigarettes, 95.8% and 92.3% of the menthol and nonmenthol smokers, respectively, chose their usual brand as their first choice. IN and HN cigarettes were significantly more likely to be ranked higher than LN cigarettes (Odds Ratios, *OR* = 2.5 (95% *CI*: 1.3–4.6); $|t| = 2.87$, $p = .005$ and 4.0 (95% *CI*: 2.1–7.5); $|t| = 4.37$, $p < .0001$, respectively). No differences between men and women were observed.

Perceived Health Risk

Participants scored the HN cigarettes as having a significantly higher risk of addiction than the LN and IN cigarettes (Table 2). Smokers rated the HN cigarettes as having significantly greater PHRs on all other measures relative to LN cigarettes. No significant differences in perception of health risks were observed for IN versus HN cigarettes, with the exception of risk for addiction. Significant differences were observed between LN versus IN cigarettes for lung cancer and approached significance for risk of emphysema ($p = .066$). No differences between men and women or by menthol status were observed.

Heart Rate and Blood Pressure

Systolic and diastolic blood pressure and heart rate were significantly higher for the HN compared to the LN cigarettes (120.2±1.6 vs. 117.1±1.6; $|t| = 2.45$, $p = .049$; 78.0±1.2 vs. 74.6±1.2, $|t| = 3.49$, $p = .002$; and 73.9±4.5 vs. 71.3±1.5, $|t| = 3.20$, $p = .006$). IN cigarettes revealed significantly higher diastolic blood pressure (77.7±1.2 vs. 74.6±1.2; $|t| = 3.24$, $p = .005$) and higher heart rate (73.4±1.5 vs. 71.3±1.5; $|t| = 2.51$, $p = .042$) than LN cigarettes. No significant differences were observed for other comparisons. No menthol status, gender, or menthol status or gender by nicotine content interaction effects were observed for blood pressure. The heart rates of nonmenthol smokers were borderline significantly higher than the heart rates of menthol smokers (75.3±1.8 vs. 70.4±2.0; $F = 3.90$, $p = .055$).

STUDY 2

Methods

Subjects and Cigarettes

Subject recruitment methods and cigarettes were identical to Study 1 with the following exceptions: subjects were only recruited at the University of Minnesota and smoked 10–20

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Table 2. Study 1: Perceived Health Risk (PHR) by Nicotine Level

Measurement (0–100 Scale)	Usual Brand Mean (*SE*)	Lower LS Mean (*SE*)[a]	Intermediate LS Mean (*SE*)[a]	Higher LS Mean (*SE*)[a]	Lower vs. Intermediate	Lower vs. Higher	Intermediate vs. Higher
Lung cancer	79.8 (2.6)	56.6 (4.6)	66.7 (4.6)	68.3 (4.6)	$\|t\| = 2.92^{+}$	$\|t\| = 3.41$*	$\|t\| = 0.48$
Emphysema	77.6 (2.7)	60.6 (4.2)	67.9 (4.2)	70.8 (4.2)	$\|t\| = 2.33$	$\|t\| = 3.26$*	$\|t\| = 0.93$
Bronchitis	76.2 (3.2)	59.4 (4.2)	65.7 (4.2)	68.4 (4.1)	$\|t\| = 1.93$	$\|t\| = 2.76^{+}$	$\|t\| = 0.82$
Other cancers	74.5 (3.0)	57.2 (3.9)	64.0 (3.9)	68.8 (3.9)	$\|t\| = 2.02$	$\|t\| = 3.43$*	$\|t\| = 1.40$
Heart disease	77.1 (3.0)	62.6 (3.9)	67.6 (3.9)	72.3 (3.9)	$\|t\| = 1.65$	$\|t\| = 3.23$*	$\|t\| = 1.57$
Risk of addiction	85.9 (2.3)	51.0 (4.8)	58.1 (4.7)	69.9 (4.7)	$\|t\| = 1.82$	$\|t\| = 4.82$***	$\|t\| = 3.00$*
Stroke	70.9 (3.3)	58.1 (3.7)	62.8 (3.6)	68.3 (3.6)	$\|t\| = 1.63$	$\|t\| = 3.56$*	$\|t\| = 1.92$

Notes. LS = least square; SE = standard error.
[a]Adjusted for baseline response (usual brand), gender, menthol use, study site, cigarette order, interactions between nicotine level and menthol and gender, and repeated measures across subjects.
$^{+}p \le .05$. *$p \le .01$. **$p \le .001$. ***$p \le .0001$.

cigarettes/day (to reduce the number of cartons of cigarettes that would be needed).

Study Design

Subjects attended three clinic visits. For the first clinic visit, subjects were instructed to continue smoking their usual cigarette brand for 1 week and provided a daily diary to record the number of cigarettes smoked per day, a container to collect cigarette butts, and a urine cup to collect a first morning void. Cigarette butts were collected by the subject on the day before the next clinic visit 1 week later (to be analyzed later) and the urine on the day of the clinic visit.

On the second clinic visit, subjects returned their daily diary, the filled container of usual-brand cigarette butts, and the first-morning-void urine sample. Heart rate, blood pressure, and CO were obtained and subjective forms similar to those in Study 1 were completed. Subjects were randomly assigned experimental cigarettes in a double-blind manner and instructed to smoke the experimental cigarette exclusively for 1 week. Subjects were given a daily diary to record the number of study and/or usual-brand cigarettes smoked each day. Subjects collected cigarette butts on the last day of study. Subjects were asked to collect a first-morning-void urine sample on the day of the third clinic visit, which involved the same procedures as the second clinic visit.

Urine samples were analyzed for total cotinine (Murphy et al., 2004) and total nicotine equivalents (TNE), which is the sum of nicotine, cotinine, trans 3′-hydroxycotinine, and their respective glucuronide conjugates (Scherer et al., 2007).

Statistical Analysis

Demographic and smoking history data were summarized. Cigarettes smoked (both usual brand and experimental) in a given week were summed over the first 7 reported days. If the number of cigarettes smoked was missing for 1 day, the average of the other days in that week was used in its place. Outcome variables similar to Study 1 were analyzed in Study 2, except biomarker levels were also assessed (CO, total cotinine, and TNE). All outcomes were analyzed using linear regression models adjusting for baseline response (relating to their usual brand), experimental cigarette nicotine content (LN, IN, HN), gender, and nicotine content and gender interaction. Change in number of cigarettes smoked, CO, and other biomarker values from usual brand (baseline) were also assessed by cigarette type. We were not able to adjust for menthol status in this study due to small numbers in the menthol group. LS means ± *SE* were reported for each nicotine level unless otherwise noted, and *p* values were adjusted for multiple comparisons using a Bonferroni correction. All significance levels were set at .05.

Results

Thirty-six subjects were randomized to LN (n = 13), IN (n = 11), and HN (n = 12) cigarettes. One subject from the LN group was excluded because he did not smoke any experimental cigarettes. Table 3 shows the demographic and smoking history information; no significant differences were observed across cigarette types, although a trend was observed for age. Because of the small sample size, only a few key significant results will be discussed and most of the results are descriptive.

Compliance With Product

The footnote for Table 4 shows the number of usual-brand cigarettes smoked by each smoker who reported using them. Among those assigned to the LN cigarettes, five subjects (including the subject who never smoked the experimental cigarette) smoked their usual-brand cigarette during the treatment period. Among those assigned to the IN and HN cigarettes, 4 and 2 subjects, respectively, smoked their usual-brand cigarettes during the treatment week.

Cigarette and Nicotine Exposure

Subjects who were assigned to the LN cigarettes smoked significantly fewer experimental cigarettes over the course of the treatment week than those assigned to the HN cigarettes (92.1 ± 15.9 vs. 157.6 ± 15.2; $|t| = 2.97$, $p = .018$; Figure 1). Additionally, those who were assigned to the HN cigarettes smoked significantly more experimental cigarettes during the treatment week than usual-brand cigarettes during the pretreatment week (change: 33.0 ± 13.4; $|t| = 2.46$, $p = .020$). Those assigned to the LN cigarettes smoked fewer compared with their usual-brand cigarettes, although this was not statistically significant (change: −10.6 ± 14.0 cigarettes/week, $|t| = -0.76$, $p = .454$). There were no differences by gender.

Table 4 shows the biomarker values for each subject, and Table 5 shows the mean values by cigarette type assignment. Comparisons by randomization group (LN, IN, HN) found no significant differences in baseline levels of CO (20.1 ± 3.0,

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Table 3. Study 2: Demographics and Smoking History of All Subjects and by Nicotine Level

	Total	Lower Nicotine	Intermediate Nicotine	Higher Nicotine	
Variables		*N*(%)			*p* Value
Total	35	12 (34.3)	11 (31.4)	12 (34.3)	
Gender					
Male	15 (42.9)	4 (33.3)	4 (36.4)	7 (58.3)	.477
Female	20 (57.1)	8 (66.7)	7 (63.6)	5 (41.7)	
Ethnicity					
Hispanic or Latino	2 (5.7)	1 (8.3)	0 (0.0)	1 (8.3)	1.00
Not Hispanic/Latino	33 (94.9)	11 (91.7)	11 (100.0)	11 (91.7)	
Race					
American Indian/Alaskan native	1 (2.9)	1 (8.3)	0 (0.0)	0 (0.0)	.585
Asian	1 (2.9)	0 (0.0)	1 (9.1)	0 (0.0)	
Black or African American	7 (20.0)	3 (25.0)	1 (9.1)	3 (25.0)	
White	22 (62.9)	8 (66.7)	7 (63.6)	7 (58.3)	
More than one race	4 (11.4)	0 (0.0)	2 (18.2)	2 (16.7)	
Smoke menthols					
No	24 (68.6)	8 (66.7)	8 (72.7)	8 (66.7)	1.00
Yes	11 (31.4)	4 (33.3)	3 (27.3)	4 (33.3)	

	N	Mean (*SD*)	*N*	Mean (*SD*)	*N*	Mean (*SD*)	*N*	Mean (*SD*)	*p* Value
Age (y)	35	37.9 (11.6)	12	37.0 (12.4)	11	32.7 (10.8)	12	43.6 (9.6)	.072
Highest grade completed	35	13.4 (2.4)	12	13.8 (1.1)	11	13.9(1.9)	12	12.6 (3.5)	.370
Cigarettes/day	35	17.2 (4.4)	12	16.8 (5.1)	11	16.1 (3.5)	12	18.7 (4.3)	.351
Years daily smoking	35	17.9 (11.0)	12	14.8 (10.9)	11	15.4 (11.7)	12	23.3 (9.0)	.104

16.4±2.8, 17.0±2.5; $F = 0.47$, $p = .629$), total cotinine (22.2±4.7, 21.0±4.5, 19.2±4.0; $F = 0.13$, $p = .881$), or TNE (90.3±15.6, 71.4±14.8, 74.4±13.5; $F = 0.45$, $p = .644$).

Among subjects who smoked three or fewer of their usual-brand cigarettes during the treatment week prior to urine collection ($n = 32$), subjects smoking the HN and IN cigarettes had higher CO levels than those smoking LN cigarettes, although this difference was only borderline significant for the IN cigarettes ($p = .068$). There were no significant differences in CO levels between the IN and HN cigarettes. In comparison with their baseline (usual brand) CO levels, those smoking the LN cigarettes had a significant decrease in CO levels (change: 7.8±2.8; $|t| = 2.77$, $p = .010$), whereas CO did not change significantly in the IN and HN conditions compared with baseline (change: −2.0±2.6; $|t| = 0.74$, $p = .465$ and −2.7±2.4; $|t| = 1.12$, $p = .273$, respectively).

Among those with available biomarker data and who smoked three or fewer usual-brand cigarettes during the treatment week ($n = 31$), total cotinine and TNE were significantly lower in subjects using the LN cigarettes than those using the HN cigarettes (Table 5). In comparison to baseline, those smoking the LN cigarettes had significantly lower total cotinine and TNE levels at the end of treatment (change: −16.2±5.0; $|t| = 3.24$, $p = .003$ and −64.5±14.5; $|t| = 4.46$, $p = .0002$, respectively), and those smoking the IN cigarettes had borderline significantly lower cotinine levels (change: −7.6±4.7; $|t| = 1.62$, $p = .117$) and significantly lower TNE levels (change: −33.0±13.5; $|t| = 2.44$, $p = .022$). Total cotinine and TNE levels among those smoking HN cigarettes were not significantly different from baseline (change: 1.3±4.4; $|t| = 0.31$, $p = .761$ and −14.7±12.7; $|t| = 1.16$, $p = .258$, respectively). There were no differences by gender.

Modified Cigarette Evaluation Questionnaire

Significant differences were observed between the LN and HN cigarette conditions for satisfaction (8.3±7.5 vs. 47.8±7.2; $|t| = 3.71$, $p = .003$) and Enjoyment of Sensation (13.8±7.5 vs. 41.3±7.0; $|t| = 2.63$, $p = .041$). A trend was observed for Psychological Reward (28.5±6.2 vs. 47.8±6.1; $|t| = 2.22$, $p = .105$). All other comparisons between nicotine content cigarette conditions and mCEQ outcomes were not statistically significant. There were also no differences by gender.

Other Subjective Responses to Spectrum Cigarettes

Smokers assigned the HN cigarettes compared with LN and IN conditions reported greater liking (45.2±7.6 vs. 7.1±8.0 and 16.7±8.1; $|t| = 3.44$, $p = .006$ and $|t| = 2.58$, $p = .046$, respectively) and significantly or nearly significantly lower disliking of the cigarettes (43.5±7.9 vs. 91.5±8.4 and 72.6±8.7; $|t| = 4.15$, $p = .001$ and $|t| = 2.49$, $p = .058$, respectively). Consistent with the subjective ratings, participants in the HN condition indicated that they would switch to money over cigarettes at a higher monetary value than subjects in the LN condition ($5.44±0.69 vs. $1.68±0.72, $|t| = -3.79$, $p = .002$). There were no differences in these outcomes by gender.

DISCUSSION

The results from these studies indicated that these research cigarettes were generally distinguishable and produced a dose–response effect. Smokers, blind to cigarette type, were able to discriminate cigarettes varying in nicotine content, particularly between the LN and HN and LN and IN doses, with the LN

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Table 4. Study 2: Levels of Urinary Cotinine and Total Nicotine Equivalents for Each Subject by Cigarette Type

	Cotinine (nmol/ml)		Total nicotine equivalent (nmol/ml)	
Cigarette type	Usual cigarettes	Experimental cigarettes	Usual cigarettes	Experimental cigarettes
Subject Number				
Lower nicotine				
202	14.0	4.7	72	17
204	6.7	0.1	58	1
214*	8.8	1.7	25	5
221*	22.6	5.0	67	16
231	41.3	0.5	123	2
232	36.3	17.0	170	69
234	16.3	0.1	62	LOD
235	17.7	8.8	115	56
238	59.7	13.7	195	38
240*	8.6	14.0	24	48
246	42.1	Missing	162	Missing
250*	14.0	0.8	31	2
Intermediate nicotine				
205*	25.3	2.4	78	8
210	25.0	6.6	83	19
211*	34.2	10.3	70	24
216*	1.3	1.9	6	12
225*	44.7	37.3	107	93
227	14.7	14.6	91	66
230	3.2	2.1	18	12
236	24.5	7.0	52	18
239	17.5	2.3	51	7
244	7.6	36.6	82	88
248	14.5	7.9	63	29
Higher nicotine				
206	3.4	4.0	41	41
209	39.8	14.1	193	45
213	20.6	24.3	65	61
215*	28.7	38.8	91	85
222	7.5	16.3	26	68
226*	10.0	23.0	94	72
233	8.1	24.1	60	58
237	29.4	11.2	104	56
242	29.5	Missing	62	Missing
245	18.3	26.6	44	69
252	21.4	14.0	51	41
254	14.5	20.3	42	62

Note. *Self-reported usual-brand cigarettes smoked during treatment period: 214 and 221 smoked usual-brand cigarettes after urine collection, 240 = 13 cigarettes, 250 = 1 cigarette, 205 = 3 cigarettes, 211 = 2 cigarettes, 216 = 7 cigarettes, 225 = 1 cigarette, 215 smoked after urine collection, 226 = 1 cigarette; LOD = limit of detection.

cigarettes producing a less favorable subjective response. Only two variables led to a distinction between the IN and HN cigarettes in the expected direction, "risk for addiction" in Study 1 and "liking" in Study 2. Cotinine, TNE, and CO levels significantly decreased during Study 2 following the LN cigarettes (compared with baseline) and TNE decreased following the IN cigarettes. No significant changes on any of these exposure measures were observed for the HN dose compared with usual brand, although these subjects smoked significantly higher number of cigarettes. Significant differences between the HN and LN conditions were observed for CO, total cotinine, and TNE.

Prior studies also observed that smokers can discriminate subjectively across differing nicotine content cigarettes (Benowitz et al., 2007, 2012; Benowitz, Jacob, & Herrera, 2006; Hatsukami, Kotlyar et al., 2010), particularly between higher versus lower nicotine content cigarettes. In an acute dosing study, subjects were asked to smoke one of their usual-brand cigarette and then on five separate occasions to smoke a research cigarette that varied in nicotine content (from 0.6 to 10.1 mg nicotine content per cigarette or 0.13 to 0.89 mg Federal Trade Commission (FTC) determined nicotine yield; Benowitz, Jacob, & Herrera 2006). Lower nicotine content cigarettes were rated as less strong, much too smooth, poorer

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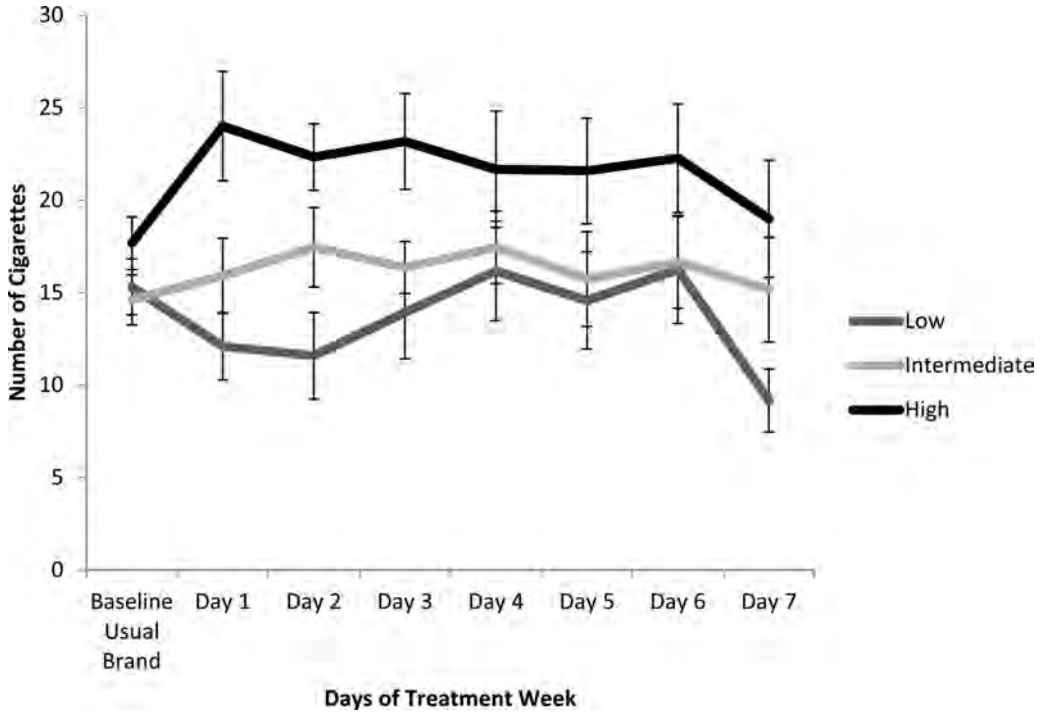


Figure 1. Means and standard errors (*SE*) of number of usual and experimental cigarettes smoked by nicotine level.

Table 5. Study 2: Biomarker Levels After 1 Week of Product Use by Nicotine Level, Adjusted for Baseline Levels and Gender

	Usual Brand	Lower LS	Intermediate LS	Higher LS			
Measurement	Mean (*SE*)	Mean (*SE*)	Mean (*SE*)	Mean (*SE*)	Lower vs. Intermediate	Lower vs. Higher	Intermediate vs. Higher
Cotinine (nmol/ml)	21.8 (2.3)	5.0 (3.5)	13.4 (3.2)	20.3 (3.1)	|*t*| = 1.77	|*t*| = 3.31*	|*t*| = 1.55
Nicotine equivalents (nmol/ml)	81.6 (7.8)	19.9 (8.1)	40.1 (7.6)	60.8 (7.1)	|*t*| = 1.81	|*t*| = 3.79*	|*t*| = 2.01
Carbon monoxide	17.1 (1.4)	11.3 (2.3)	18.9 (2.1)	19.9 (1.9)	|*t*| = 2.43	|*t*| = 2.91**	|*t*| = 0.38

Notes. LS = least square; *SE* = standard error.
Excludes three subjects who smoked more than three usual-brand cigarettes during the study period.
*$p \leq .01$. **$p \leq .05$.

quality, less satisfying, and having less nicotine. The greatest differences were observed with the 1 mg (0.13 mg nicotine yield) compared with 8 mg (0.63 mg nicotine yield) and 12 mg (0.89 mg nicotine yield) nicotine content cigarettes. The 2 mg (0.18 mg nicotine yield) and 4 mg (0.33 nicotine yield) nicotine content cigarettes occasionally showed differences compared with the higher nicotine content cigarettes. No differences were observed among 1, 2, and 4 mg nicotine content cigarettes (<0.33 nicotine yield). In another study, Benowitz and colleagues (2007) examined the effects of a progressive weekly reduction in nicotine content in cigarettes (12, 8, 4, 2, and 1 mg nominal nicotine content or 0.8, 0.6, 0.3, 0.2, and 0.1 mg FTC determined nicotine yield). With regards to subjective measures, no change was observed in the Profile of Mood Scale score or Center for Epidemiologic Studies Depression Scale depression rating. However, withdrawal scores for irritability and increased eating were significantly higher at week 6 while smoking 1 mg nicotine content cigarette (0.1 mg nicotine yield) compared with baseline while smoking usual brand. No direct comparisons were described between the different doses of cigarettes for these measures and measures of cigarette acceptance, although subjects reported that the reduced-nicotine cigarettes were less strong, less flavorful, of generally lower quality and less satisfying compared with their usual-brand cigarettes. The third study involved a progressive reduction in nicotine content in cigarettes at monthly intervals using similar nicotine content cigarettes as the prior weekly reduction study (0.9, 0.6, 0.4, 0.2, and 0.1 mg nicotine yield). Increased confusion and decreased vigor were observed when smokers switched from their usual-brand cigarettes to cigarettes that were equal to or less than 4 mg nicotine content (or 0.4 mg nicotine yield). Similar to the prior study, the reduced-nicotine content cigarettes were rated as milder, less satisfying, having lower nicotine effect, and of lesser quality than usual-brand cigarettes. No other details comparing the varying nicotine content cigarettes were provided. In a study conducted by Hatsukami, Kotylar, and colleagues (2010), rather than a progressive reduction in nicotine content, subjects were asked to switch completely to

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0.05 or 0.3 mg nicotine yield cigarettes or to nicotine lozenge for 6 weeks. Risk for addiction was perceived to be significantly lower for the 0.05 mg compared with the 0.3 mg nicotine cigarette. The results from these previous studies are similar to our finding that the greatest differences in subjective responses occur between a very LN content cigarette (most likely <0.1 mg nicotine yield) compared with a substantially higher nicotine content cigarette (>0.3 or 0.4 mg nicotine yield).

Reductions in smoking behavior and exposure to cotinine, nicotine, and CO is typically observed with the lower nicotine content cigarettes. In the acute cigarette dosing study conducted by Benowitz and colleagues (2006), a significant dose–response relationship was observed between intake of nicotine and machine determined nicotine yield (and nicotine content), although actual exposure was generally greater than predicted from machine determined nicotine yield of the cigarettes. Compensation was lower in the 1 mg cigarette (0.13 mg nicotine yield) compared with the 4 mg cigarette (0.33 nicotine yield; 19% vs. 38%, respectively) and to the 8 mg (0.63 mg nicotine yield) nicotine cigarette (64%); although these differences were not significant, they suggest that compensation may be observed less at very low yields. In the Benowitz and colleagues (2007) study where subjects underwent a weekly progressive decrease in nicotine content, no significant change was observed for smoking behavior or CO during nicotine reduction, but a progressive decrease occurred for cotinine levels. In the Benowitz and colleagues (2012) study where subjects underwent a monthly decrease in nicotine content, no significant change in smoking behavior was observed until smokers were switched to 1 mg nicotine content cigarette (or 0.1 mg nicotine yield) at which point smoking rate declined. A significant decrease in cotinine was observed after switching to 4 mg (or 0.4 mg nicotine yield) nicotine cigarette. By the end of the study at week 26 (1 mg nicotine content), cotinine levels were 30% of the baseline value among those who complied with use of the cigarettes. With regard to the Hatsukami, Kotlyar, and colleagues (2010) study, where subjects reduced to lower nicotine content cigarettes immediately, an increased number of cigarettes and CO were observed for the 0.3 mg nicotine yield cigarette relative to baseline, but these measures decreased for the 0.05 mg cigarette, resulting in significant or near significant differences between the two cigarette yields. Cotinine was significantly reduced in both cigarette conditions with greatest reductions in those assigned to the 0.05 mg nicotine cigarette. The results from these studies are concordant with our findings that smokers are sensitive to nicotine contents of the cigarettes and tend to show decreases in smoking behavior and exposure at the lowest nicotine content. Furthermore, significant changes in subjective responses are also likely to occur at nicotine yields <0.1 mg. Increased smoking with or without increases in exposure (e.g., CO) may occur at more intermediate doses of nicotine, but whether this increase has a significant impact on health is unknown and will require more investigation.

Three additional results are of interest. Less compliance was observed with the LN and IN than HN cigarettes. These results are similar to those observed by Hatsukami, Kotylar, and colleagues (2010), in which the lower dose was modestly associated with more subjects smoking usual-brand cigarettes. With regards to differences in menthol versus nonmenthol cigarettes, smokers of menthol cigarettes did not report satisfaction or liking their cigarettes as much as the nonmenthol smokers. It is possible that switching to cigarettes that differed in both menthol and nicotine content levels compared with their usual brands led to more dissatisfaction with these cigarettes or that menthol smokers tended to smoke higher nicotine content cigarettes. Efforts to manufacture menthol cigarettes that are equally palatable as nonmenthol cigarettes may be important. Finally, gender differences were only observed in Study 1 and for craving reduction and monetary value of cigarettes. Although these results are suggestive, due to the small sample size, further research is required before any conclusions can be made.

In summary, this study showed that the dose–response results with the Spectrum research cigarettes are similar to those observed in prior studies that compared cigarettes varying in nicotine content. In general, very LN content cigarettes (especially <0.1 mg nicotine yield) tend to lead to reduced smoking and significant differences in subjective responses compared with cigarettes with higher nicotine yields (>0.4 or 0.3 mg nicotine yield cigarettes).

FUNDING

This work was supported by the National Institute on Drug Abuse at the National Institutes of Health Intramural Research Program and under contract no. HHSN271201000003C, U54DA031659, and the University of Minnesota, Forster Family Professor in Cancer Prevention Endowment.

DECLARATION OF INTERESTS

Dorothy Hatsukami was funded by Nabi Biopharmaceuticals and NIDA to be a site for a nicotine immunotherapy trial. There are no other declarations.

ACKNOWLEDGMENTS

We appreciate the contributions of Kathy Longley for her assistance on the manuscript. Special thanks to Drs. Kenneth Davis and Poonam Pande at RTI for their role in making these cigarettes available to researchers.

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Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes (P1S1)

This study is currently recruiting participants.

Verified August 2013 by University of Pittsburgh

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Information provided by (Responsible Party):
University of Pittsburgh

ClinicalTrials.gov Identifier:
NCT01681875

First received: August 30, 2012
Last updated: August 29, 2013
Last verified: August 2013
History of Changes

Full Text View | Tabular View | No Study Results Posted | Disclaimer | How to Read a Study Record

Purpose

Project 1, Study 1 will evaluate the relationship between nicotine yield of very low nicotine content cigarettes and cigarettes smoked per day, nicotine exposure, discomfort/dysfunction, other health-related behaviors, nicotine/tobacco dependence, biomarkers of tobacco exposure, intention to quit, compensatory smoking, other tobacco use, cigarette characteristics, cognitive function, cardiovascular function, and perceived risk. We will also consider differences between conditions in compliance with product use.

Condition	Intervention
Tobacco Dependence	Other: very low nicotine content cigarettes

Study Type: Interventional

Study Design: Allocation: Randomized
Intervention Model: Parallel Assignment
Masking: Double Blind (Subject, Investigator, Outcomes Assessor)
Primary Purpose: Basic Science

Official Title: Project 1, Study 1: Investigating the Impact of Nicotine Using Spectrum Cigarettes

Resource links provided by NLM:

MedlinePlus related topics: Smoking

Drug Information available for: Nicotine tartrate Nicotine polacrilex

U.S. FDA Resources

Further study details as provided by University of Pittsburgh:

Primary Outcome Measures:

- Number of cigarettes smoked per day [Time Frame: End of 6 week intervention] [Designated as safety issue: No]

Estimated Enrollment: 840
Study Start Date: June 2013
Estimated Study Completion Date: July 2014
Estimated Primary Completion Date: July 2014 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
Experimental: 0.8 mg nicotine with 9 mg tar very low nicotine content cigarettes SPECTRUM Cigarette: 0.8 (±0.15) mg nicotine with 9 (±1.5) mg tar (standard nicotine and tar yields of commercially-available cigarettes; control condition)	Other: very low nicotine content cigarettes
Experimental: 0.26 mg nicotine with 9 mg tar very low nicotine content cigarettes SPECTRUM Cigarette: 0.26 (±0.06) mg nicotine with 9 (±1.5) mg tar	Other: very low nicotine content cigarettes
Experimental: 0.12 mg nicotine with 9 mg tar very low nicotine content cigarettes SPECTRUM Cigarette: 0.12 (±0.03) mg nicotine with 9 (±1.5) mg tar	Other: very low nicotine content cigarettes
Experimental: 0.07 mg nicotine with 9 mg tar very low nicotine content cigarettes SPECTRUM Cigarette: 0.07 (±0.02) mg nicotine with 9 (±1.5) mg tar	Other: very low nicotine content cigarettes
Experimental: 0.03 mg nicotine with 9 mg tar very low nicotine content cigarettes SPECTRUM Cigarette: 0.03 (±0.01) mg nicotine with 9 (±1.5) mg tar	Other: very low nicotine content cigarettes
Experimental: 0.04 mg nicotine with 13 mg tar very low nicotine content cigarettes SPECTRUM Cigarette: 0.04 (±0.02) mg nicotine with 13 (±2) mg tar	Other: very low nicotine content cigarettes
Usual brand very low nicotine content cigarettes Usual brand cigarettes (control condition)	Other: very low nicotine content cigarettes

Eligibility

Ages Eligible for Study: 18 Years and older
Genders Eligible for Study: Both
Accepts Healthy Volunteers: No

Criteria
Inclusion Criteria:

- Age 18+
- Smoke an average of at least five cigarettes per day for at least 1 year
- Breath CO levels > 8 ppm (if ≤ 8 ppm, then NicAlert Strip > 2)

Exclusion Criteria:

- Intention to quit smoking in the next 30 days
- Currently seeking treatment for smoking cessation
- Currently using nicotine replacement therapies or other pharmacotherapies as cessation aid (intermittent use acceptable)
- A quit attempt in the past 30 days resulting in greater than 3 days of abstinence

- Using other tobacco products more than 9 days in the past 30 days
- Significant unstable medical conditions (Any significant change in a serious medical condition occurring during the past 3 months including, cardiovascular disease, COPD, and cancer, as determined by the licensed medical professional at each site)
- Significant unstable psychiatric conditions (Any significant change in psychiatric symptoms during the past 3 months as determined by the licensed medical professional at each site)
- Schizophrenia and schizoaffective disorder
- Psychiatric medication changes in the past 3 months
- Positive toxicology screen for any of the following drugs: cocaine, opiates, methadone, benzodiazepines, barbiturates, amphetamines, methamphetamines, and PCP
    a. Marijuana will be tested for but will not be an exclusionary criterion.
    b. Participants with valid prescriptions for opiates, benzodiazepines, barbiturates, amphetamines or methadone will not be excluded.
    c. Participants failing the toxicology screen will be allowed to re-screen once.
- Blood alcohol level > 0.01 a. Participants failing the blood alcohol screen will be allowed to re-screen once.
- Binge drinking alcohol (more than 9 days in the past 30 days, 4/5 in a 2 hour period(female/male))
- Pregnant or breastfeeding
- Smoking 'roll your own cigarettes' exclusively
- Currently taking anticonvulsant medication
- CO reading >80 ppm
- Systolic BP greater than or equal to 160 a. Participants failing for blood pressure will be allowed to re-screen once.
- Diastolic BP greater than or equal to 100 a. Participants failing for blood pressure will be allowed to re-screen once.
- Systolic BP below 90 a. Participants failing for blood pressure will be allowed to re-screen once.
- Diastolic BP below 50 a. Participants failing for blood pressure will be allowed to re-screen once.
- Heart rate greater than or equal to 115bpm a. Participants failing for heart rate will be allowed to re-screen once.
- Heart rate lower than 45bpm a. Participants failing for heart rate will be allowed to re-screen once.
- Indicating any suicidal ideation in the past month or suicide attempts in the past 10 years
- Inability to independently read and comprehend the consent form and other written study materials and measures
- Having participated in a research study during the past three months in which the participant:
    a. Smoked a cigarette that was not his/her usual brand cigarette for more than one day
    b. Used any tobacco products beyond normal use for more than one day
    c. Used any nicotine replacement products or smoking cessation medications for more than one day

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Please refer to this study by its ClinicalTrials.gov identifier: NCT01681875

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More Information

Publications:

Donny EC, Jones M. Prolonged exposure to denicotinized cigarettes with or without transdermal nicotine. Drug Alcohol Depend. 2009 Sep 1;104(1-2):23-33. Epub 2009 May 15.

Hatsukami DK, Kotlyar M, Hertsgaard LA, Zhang Y, Carmella SG, Jensen JA, Allen SS, Shields PG, Murphy SE, Stepanov I, Hecht SS. Reduced nicotine content cigarettes: effects on toxicant exposure, dependence and cessation. Addiction. 2010 Feb;105(2):343-55.

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Responsible Party:	University of Pittsburgh
ClinicalTrials.gov Identifier:	NCT01681875 History of Changes
Other Study ID Numbers:	U54 DA031659, U54DA031659-01
Study First Received:	August 30, 2012
Last Updated:	August 29, 2013
Health Authority:	United States: Food and Drug Administration United States: Data and Safety Monitoring Board

Keywords provided by University of Pittsburgh:
Biomarkers of exposure
Compensatory smoking
Nicotine dependence
Reduced nicotine cigarettes
Tobacco withdrawal

Additional relevant MeSH terms:

Tobacco Use Disorder	Pharmacologic Actions
Substance-Related Disorders	Nicotinic Agonists
Mental Disorders	Cholinergic Agonists
Nicotine	Cholinergic Agents
Nicotine polacrilex	Neurotransmitter Agents
Ganglionic Stimulants	Molecular Mechanisms of Pharmacological Action
Autonomic Agents	Central Nervous System Stimulants
Peripheral Nervous System Agents	Central Nervous System Agents
Physiological Effects of Drugs	Therapeutic Uses

ClinicalTrials.gov processed this record on September 19, 2013

Psychopharmacology (2011) 215:379 389
DOI 10.1007/s00213 010 2144 9

ORIGINAL INVESTIGATION

The effect of nicotine and trauma context on acoustic startle in smokers with and without posttraumatic stress disorder

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Received: 25 March 2010 / Accepted: 10 December 2010 / Published online: 29 December 2010
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Abstract
Rationale Exaggerated startle response is a prominent feature of posttraumatic stress disorder (PTSD) although results examining differences in the acoustic startle response (ASR) between those with and without PTSD are mixed. One variable that may affect ASR among persons with PTSD is smoking. Individuals with PTSD are more likely to smoke and have greater difficulty quitting smoking. While smokers with PTSD report that smoking provides significant relief of negative affect and PTSD symptoms, the effects of smoking or nicotine deprivation on startle reactivity among smokers with PTSD are unknown.

Objectives The purposes of the current study were to (1) examine baseline acoustic startle response (ASR) in smokers with and without PTSD under conditions of overnight abstinence, (2) evaluate the effect of smoking on ASR, and (3) evaluate the contextual effects of trauma versus neutral script presentations.
Methods ASR was measured among 48 smokers with and without PTSD in the context of a 2 (group: PTSD vs. non-PTSD)×2 (context: trauma vs. neutral)×3 (smoking condition: usual brand cigarette vs. denicotinized cigarette vs. no smoking) design.
Results Effects of modest size indicated that (1) PTSD participants demonstrated higher ASR (2) compared to non-PTSD participants, PTSD participants reported greater negative affect following a trauma-related script, and (3) following a trauma-related script and smoking a usual brand cigarette, PTSD participants demonstrated higher ASR.
Conclusions Although many smokers with PTSD report that smoking reduces PTSD symptoms, results suggest that smoking may actually potentiate or maintain an exaggerated startle response.

Keywords Acoustic startle response · Posttraumatic stress disorder · Nicotine · Cigarette smoking

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Posttraumatic stress disorder (PTSD) is a prevalent psychiatric disorder estimated to occur in approximately 10% of the United States population. Psychophysiological symptoms have long been a prominent feature of trauma-related disorders (Kardiner 1941), and exaggerated startle response has been codified as PTSD arousal criterion D5 in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (American Psychiatric Association 1994). Startle is characterized by a pattern of muscular reflexes

that is produced by a sudden or unexpected intense stimulus (Orr and Roth 2000). In humans, the acoustic startle response (ASR) represents a reflexive response to a high intensity, abrupt auditory stimulus and is commonly measured as a change in electromyogram (EMG) activity resulting from contraction of the orbicularis oculi muscle.

Over 25 studies have quantified ASR magnitude across a variety of PTSD populations (for a review, see Metzger et al. 1999). While meta-analyses of studies comparing the magnitude of startle responses between individuals with and without PTSD provide validation of the DSM-IV PTSD symptom of exaggerated startle, there is significant variation across studies, and many studies have failed to provide evidence for exaggerated startle among those with PTSD (Metzger et al. 1999; Pole 2007).

In a review of this literature, Grillon and Baas (2003) explored the complexity of the PTSD startle data and suggested a number of variables (e.g., presence of medications, length of PTSD duration, and contextual effects) that may moderate ASR. While a number of studies have suggested that ASR in PTSD participants may be modulated by aversive states (for a review, see Grillon and Baas 2003; Pole 2007), a more recent meta-analysis found no evidence that startle effect sizes were significantly related to whether the study involved a threat condition or other potential moderators including whether or not patients with psychoactive medications were excluded, PTSD symptom severity, trauma types, and types of startle stimuli (Pole 2007).

One potential variable that may affect magnitude of ASR among persons with PTSD that has not been previously evaluated is smoking and nicotine. Smoking is highly prevalent among those with PTSD (Beckham 1999; Lasser et al. 2000) with rates as high as 60% (Beckham et al. 2007). No previous PTSD startle studies have reported smoking rates among participants. While smokers with PTSD report that smoking significantly reduces PTSD symptoms (Beckham et al. 2007), the effects of smoking on ASR among individuals with PTSD who are also regular smokers have not been examined.

Relatively few studies have examined the effects of nicotine on startle magnitude, and those that have examined the effects of nicotine on the ASR have primarily been conducted in the context of examining the effects of nicotine on emotional reactivity. It is well established that startle magnitude is modulated by affective processing. Response to a startling acoustic probe is enhanced when participants view unpleasant or negative emotional pictures in comparison with pleasant or neutral pictures (e.g., Vrana et al. 1988; Cinciripini et al. 2006), while startle is reduced during viewing of positive cues (Cuthbert et al. 1996). Startle potentiation by unpleasant stimuli is thought to reflect activation of the defensive motivational system (Lang et al. 1990).

The effects of nicotine on startle modulation among smokers unassessed for psychiatric disorders have been mixed. Some studies have found no effect for nicotine deprivation on neutral context (i.e., no affective stimuli) startle responding (Kumari et al. 1997; Mueller et al. 1998) or to ASR in the presence of negative cues (Geier et al. 2000; Hogle and Curtain 2006; Piper and Curtin 2006). In contrast, other studies have reported that nicotine deprivation among smokers increased ASR across all types of stimuli (Piasecki et al. 2002), and nicotine suppresses ASR to smoking and affective cues in non-psychiatric smokers (Cinciripini et al. 2006).

Data from our laboratory indicate that following exposure to trauma-related cues, smokers with PTSD report more smoking craving, negative affect, and PTSD symptoms (Beckham et al. 2007), and smoking results in significant relief of negative affect and PTSD symptoms (Beckham et al. 2007). Ambulatory data from our research group also indicate that smokers with PTSD are more likely to ad lib smoke following the occurrence of negative affect or a PTSD symptom (Beckham et al. 2005; Beckham et al. 2008). These data suggest that smokers with PTSD may be particularly reactive to specific contexts and their craving to smoke and behavior are related to their symptomatology. The effects of smoking or nicotine deprivation on startle reactivity among individuals with PTSD, however, are unknown.

The objective of the current pilot study was to evaluate the effects of nicotine deprivation and trauma-related imagery on ASR among smokers with PTSD. While the majority of the work examining affective modulation of startle has relied on a picture-presentation paradigm (e.g., Vrana et al. 1988), the current study aimed to extend this work using a script-driven imagery paradigm (Pitman et al. 1987). Script-driven imagery has been shown to significantly differentiate PTSD from non-PTSD participants in terms of both valences (e.g., ratings of fear, anxiety, and guilt), arousal (e.g., heart rate, skin conductance; Orr et al. 1993), and smoking craving (Beckham et al. 2004). Using a 2 (group: PTSD vs. non-PTSD)×2 (context: trauma vs. neutral)×3 (smoking condition: usual brand cigarette vs. denicotinized cigarette vs. no smoking) design, the purposes of the study were to examine in smokers with and without PTSD: (1) baseline ASR under conditions of overnight abstinence, (2) the effects of smoking and nicotine on ASR, and (3) the effects of context (trauma vs. neutral) alone and in combination with smoking. We included a denicotinized cigarette condition in order to evaluate the sensory and behavioral aspects of smoking separate from the effects of nicotine. Specifically, we hypothesized that: (1) ASR would be significantly higher in participants with PTSD, (2) nicotine deprivation would potentiate the ASR, and (3) a PTSD×context

interaction where smokers with PTSD would demonstrate (a) increased PTSD symptoms and negative affect, and (b) potentiated ASR in a trauma script condition compared to a neutral script condition.

Method

Participants

Participants were 18 smokers with PTSD and 30 smokers without PTSD. Participants were eligible if they were generally healthy, not currently seeking treatment for nicotine dependence, and current cigarette smokers, reporting consumption of at least 15 cigarettes/day with afternoon expired CO concentrations of at least 15 ppm.

Participants were excluded if they had major unstable medical problems or if they used any other forms of nicotine. Candidates who met DSM-IV criteria for schizophrenia, current manic episode, lifetime but not current PTSD, or current substance abuse/dependence were excluded. Since depression is independently associated with increased smoking (Kassel et al. 2003; Kassel and Unrod 2000) and can affect startle (Grillon and Baas 2003), smokers with current major depressive disorder were excluded.

Based on the summary provided by Grillon and Baas (2003) regarding which medications affect startle response, individuals who were prescribed benzodiazepines (including diazepam, alprazolam, lorazepam, and oxazepam), clonidine, or amitriptyline were excluded. Individuals who exhibited deficits in hearing within the 500 to 3,000 Hz range as assessed through audiologic screening with a Welch Allyn device were excluded (Grillion et al. 1998).

Trauma history and PTSD diagnosis were evaluated using the Clinician-Administered PTSD Scale (CAPS; Blake et al. 1995) and the Trauma Life Events Questionnaire (Kubany et al. 2000). The Structured Clinical Interview for DSM-IV Diagnosis (First et al. 1994) was administered to assess other Axis I disorders (kappa across nine raters for diagnoses was .96).

Smoking history, nicotine dependence, and symptom measures

In addition to a general smoking history questionnaire, participants completed the Fagerström Test of Nicotine Dependence (FTND; Heatherton et al. 1991) in order to assess nicotine dependence. PTSD and mood symptoms were measured using the Davidson Trauma Scale (DTS; Davidson et al. 1997) and the Positive Affect and Negative Affect Schedule (PANAS; Watson et al. 1988a; Watson et al. 1988b). The DTS is a commonly used self-report measure of PTSD symptom severity with excellent reliability and validity (Davidson et al. 1997; McDonald et al. 2009). From the DTS, a score for re-experiencing, avoidance, and numbing, and hyperarousal symptoms were calculated for each measurement occasion. The PANAS is a 10-item positive affect and 10-item negative affect scale designed to measure positive and negative affect with demonstrated reliability and validity (Watson et al. 1988a; 1988b).

Tobacco use biomarkers and denicotinized cigarettes

Expired carbon monoxide (CO) concentrations were monitored at various times over the course of the study to (a) verify smoking status and (b) verify compliance with study requirements (e.g., overnight abstinence); CO was measured using a handheld CO monitor (piCO Smokerlyzer, Bedfont Scientific, Ltd., UK). The criterion for overnight abstinence was based on a previously validated formula (Rose and Behm 2004) that takes into account baseline CO levels. In instances in which overnight abstinence was not confirmed, participants were rescheduled to attend the study visit after completing overnight abstinence. Quest brand denicotinized cigarettes that contain at most 0.05 mg of nicotine per cigarette were used in the nicotine-free condition. Participants received either a regular or menthol Quest cigarette depending on their normal smoking brand.

Screening and script development

Following a screening session (for which participants were paid US $65), eligible participants completed a script development session and received US $100. Scripts were developed as outlined by the Pitman research group (Orr et al. 1990; Orr et al. 1993; Pitman et al. 1990; Pitman et al. 1987; Pitman et al. 1989; Shalev and Rogel-Fuchs 1993) and as used in our previous work on smoking and PTSD (Beckham et al. 2007). Six individualized scripts portraying actual experiences from the smoker's past were composed, including the three most traumatic events the participant could recall, as well as three neutral experiences. The use of three trauma and neutral scripts allows responses to be pooled across sessions for subsequent analyses.

Experimental sessions

Table 1 outlines the procedures used in each of the six experimental sessions (scheduled approximately 3 days apart) that crossed the two trauma context conditions and three smoking conditions. Payment for each session was US $100. Sessions began between 8 and 9 a.m. Smokers were required to be overnight-abstinent from cigarette smoking, alcohol, and marijuana. Breath samples were used to assess compliance. Participants then completed initial measurements and symptom questionnaires (PTSD

Table 1 Experimental nicotine abstinence/smoking challenge sessions

Time (min)	Procedures
0 15	Compliance monitoring (e.g., CO measurement, breath alcohol); PTSD symptom, and mood questionnaires
15 25	Breakfast
25 30	Attach sensors and demonstration
30 40	Baseline ASR: 1 block of 10 trials
40 45	Smoking condition: usual brand, Denicotinized cigarette or no smoking
46	Script driven imagery: trauma or neutral scripts
47 48	ASR measurement: 1 block of 3 trials
49	Repeat script
50 51	ASR measurement: 1 block of 3 trials
52 53	PPI measurement
54 60	CO measurement; PTSD symptom, and mood questionnaires

symptoms and mood). Following the initial assessment, participants were offered a standardized breakfast, and sensors necessary for the EMG were attached.

One block of 10 baseline trials of ASR was collected. Participants then completed one of three smoking conditions (nicotine, denicotinized, and no cigarette). Participants were asked to smoke a cigarette (usual brand or denicotinized) through a smoking topography device (Plowshare Technologies, Inc.; Rose et al. 1983). In the no-cigarette condition, participants were instructed to sit quietly for 5 min. Following the smoking condition, scripts were presented. Three of the sessions involved presentation of a neutral script and three included presentation of a trauma script. Smoking condition and script type were counterbalanced with the order randomly assigned.

Next, ASR was measured using two blocks of three trials. After the first block of three trials, the same script was presented again followed by the second block of three ASR trials. Participants were instructed to keep their eyes open, listen carefully, and to imagine the scene as it was described as the scripts were presented. Participants then completed a series of pre-pulse inhibition (PPI) trials. For all sessions, participants completed a final set of symptom response questionnaires (e.g., craving, mood, and PTSD symptoms) and a breath CO to verify smoke inhalation. All testing took place in a ventilated, sound attenuated room outfitted with visual and auditory participant monitoring systems.

Assessment of acoustic startle response

Electromyographic (EMG) activity of the orbicularis oculi muscle was used as an index of the ASR. The ASR task was presented binaurally through headphones (Sony model MDR-V600). All stimuli were broadband white noise. The startle stimulus was 100 dB (A; 50 ms; instantaneous rise time). EMG was collected from Ag/AgCl electrodes filled with isotonic gel placed on the skin above the orbicularis oculi, directly under the pupil and 10 mm lateral. A grounded electrode was attached to the forehead. Signal was online-amplified (×1,000) and bandpass-filtered (5 kHz to 1.0 Hz). Signals were digitized at 250 Hz, passed to a PC-based BIOPAC MP100 data acquisition workstation, and saved to disk. All auditory stimuli (ASR task and scripts) were digitally recorded and/or generated using Adobe Audition (formally Cool Edit Pro). Stimuli were presented using a computer soundcard and amplified using a low-noise sound studio quality headphone amplifier. Signal levels were calibrated to a standardized tone prior to each session with a sound pressure meter fitted with a headphone adapter.

Data reduction and analyses

Raw EMG data were integrated (root mean square method) prior to scoring. Responses were considered valid if (1) the trial was scorable (i.e., high signal/noise ratio; no artifact) and (2) a deflection was observed in 20–120 ms following startle onset. Mean baseline (25 ms pre-startle onset) amplitude and peak (i.e., maximum) response amplitude were recorded for all valid trials, and all responses were baseline-corrected. All participants were included in the analyses.

Mean startle response amplitude was modeled as the primary outcome measure. Response amplitudes for the 10 baseline habituation trials were averaged at each of the six sessions. For response habituation analyses, responses from each trial were averaged across the six sessions to yield 10 time points. To assess response amplitudes during the experimental phase (i.e., in response to smoking and script manipulations), ASR responses were averaged across the two blocks of three startle probes within each visit.

As startle data were not normally distributed, data were log-transformed before analysis. Hypotheses examining startle amplitude were tested using a series of longitudinal regression models using SAS PROC GENMOD to estimate generalized estimating equations under an assumed Gaussian distribution; working correlation matrices were specified as exchangeable. In analyses of the experimental phase, responses from the habituation trials were included as a covariate. This approach has several methodological advantages over the computation of a difference score between blocks (Mulligan and Wiesen 2003) which is an alternative method to assess adaptation across blocks (Cinciripini et al. 2006). Because of overfitting concerns, modeling was restricted to the three primary design variables: diagnostic status (PTSD versus control), smoking condition (abstinence, denicotinized, or usual brand), and script (trauma versus neutral), with response amplitude from the habituation phase and time since trauma as the only

covariates. For each outcome measure, three models were estimated: main effects, first-order (all two-way interactions) and second-order (the three-way interaction) interactions. In some instances, specified contrasts were tested using appropriate post hoc procedures. Models presented below represent the highest order of model significance. Rate (habituation) models were estimated as above using longitudinal regression for the 10-trial baseline data. The model included a covariate for trial, a proxy variable denoting diagnostic status, and an interaction term crossing the latter two. Our interest was in determining if the rate of response attenuation occurred differentially by diagnostic status (i.e., did the coefficient associated with the interaction term between trial and diagnosis differ significantly from zero).

Analyses of self-report measures including indices of PTSD symptoms and affect were conducted using SAS PROC MIXED. All analyses were conducted with SAS version 9.1. Given uneven distribution of males and females across groups, Wilcoxon nonparametric tests were used to examine whether gender was associated with startle magnitude. Results indicated that gender was not associated with ASR, $z=1.03$, n.s.

Results

Participant characteristics

Characteristics of the cohort at baseline are presented in Table 2. Gender was the only demographic factor differing between the cohorts with a substantially higher proportion of female participants in the PTSD group. For descriptive purposes, Table 2 also includes a categorization of primary traumas identified by each group during the CAPS interview.

Baseline startle

Group differences in baseline ASR responses and rates of habituation across the 10 trials are presented in Fig. 1. Results indicated that the covariate of time since trauma

Table 2 Participant demographic, diagnostic, and smoking measures

Variable	PTSD ($N=18$)	Control ($N=30$)	Test statistic
Age [M (SD)]	4.61 (10.58)	42.53 (8.72)	$F(1,46) = 0.54$, n.s.
SES (Hollings)	60.44 (9.02)	59.59 (9.66)	$F(1,45) = 0.09$, n.s.
Education [M (SD)]	11.39 (1.91)	12.63 (2.53)	$F(1,46)=3.24$, n.s.
Gender (% female)	56% (10 of 18)	17% (5 of 30)	$\chi_1^2=7.92$, $p=0.005$
Race			
% African American	67% (12 of 18)	83% (25 of 30)	
% Minority	67% (12 of 18)	87% (26 of 30)	$\chi_1^2=2.73$, n.s.
% Married	18%	23%	$\chi_1^2=0.21$, n.s.
% Veteran	33% (6 of 18)	37% (11 of 30)	$\chi_1^2=0.05$, n.s.
% Currently employed	56% (10 of 18)	33% (10 of 30)	$\chi_1^2=2.29$,n.s.
PTSD DTS	59.78 (33.41)	14.83 (17.90)	36.99, $p<.0001$
Months since trauma	202.86 (185.58)	183.23 (150.61)	$F(1,42)=0.15$, n.s.
Trauma types			
None	0% (0 of 18)	13% (4 of 30)	$\chi_7^2=12.96$, n.s.
Death/illness of loved one	28% (5 of 18)	27% (8 of 30)	
Childhood sexual abuse	28% (5 of 18)	7% (2 of 30)	
Childhood physical abuse	0% (0 of 18)	7% (2 of 30)	
Combat related traumas	17% (3 of 18)	4% (1 of 30)	
Adult physical violence	17% (3 of 18)	23% (7 of 30)	
Adult sexual assault	6% (1 of 18)	0% (0 of 30)	
Motor vehicle accident	6% (1 of 18)	20% (6 of 30)	
% MDD (lifetime)	72%	17%	$\chi_1^2=14.81$, $p=0.0001$
CO level at screen	22.72 (7.83)	25.67 (12.75)	$F(1,46)=0.78$, n.s.
Number of Cigs	19.94 (5.55)	20.79 (6.97)	$F(1,46)=0.19$, n.s.
FTND	6.61 (1.46)	6.13 (1.98)	$F(1,46)=0.79$, n.s.
PreCO	9.47 (5.26)	12.43 (6.30)	$F(1,46)=2.80$, n.s.
PostCO	12.63 (5.28)	15.32 (6.13)	$F(1,46)=2.40$, n.s.

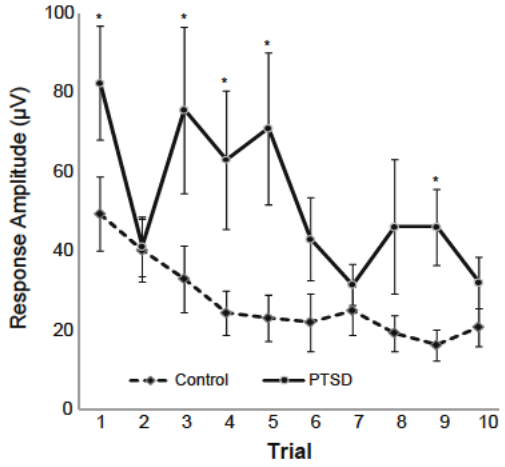


Fig. 1 Baseline startle and habituation by group. *Error bars* indicate ±1 standard deviation. *$p<0.005$

was significantly related to magnitude of ASR across trials ($\chi_1^2=4.24$; $p=0.039$) where longer time since trauma was associated with a decreased startle response. As predicted, response amplitude averaged over trials was significantly higher for participants with a diagnosis of PTSD ($\chi_1^2=7.79$; $p < 0.005$). Analyses examining habituation revealed a main effect of trials averaged over diagnosis ($\chi_1^2=27.12$; $p < 0.0001$) with ASR response attenuating at an approximate rate of 11% per trial. The interaction term crossing diagnosis with trials, however, was not statistically significant ($\chi_1^2=1.56$; $p=0.2114$), indicating that the rate of habituation did not differ significantly between those with and without PTSD.

Experimental phase: script effects on affect and PTSD symptoms

Analyses first examined the effects of the script-driven imagery procedure on PTSD symptoms and negative affect. Three separate models were estimated testing respectively for main effects, first-order interactions, and second-order interactions for each of the self-report outcomes. In no instance was model fit significantly improved by the addition of a term denoting the three-way interaction between group, context, and smoking condition. In only two instances was model fit improved by the addition of a two-way interaction term as described below.

Examining ratings of negative affect from the PANAS, results indicated a significant PTSD×context interaction. As shown in Table 3, there was no difference in levels of negative affect between neutral and trauma contexts among non-PTSD participants. Among PTSD participants, however, the trauma context was associated with greater negative affect.

Analyses examining PTSD symptom clusters revealed a main effect for group in all three PTSD symptom clusters (see Table 3) where as expected, smokers with PTSD reported higher PTSD symptoms than smokers without PTSD. Additionally, PTSD re-experiencing symptoms (DSM-IV Cluster B) were significantly higher in the trauma script condition than in the neutral condition. There was a similar but non-significant effect for PTSD avoidance symptoms (DSM-IV Cluster C), where avoidance symptoms tended to be higher in the trauma script condition in comparison to the neutral condition. Results also revealed a PTSD×smoking interaction for PTSD Cluster B symptoms, where symptoms were lower in the denicotinized condition compared to the abstinence condition, $t(225)=-2.3$, $p=0.02$.

Experimental effects on acoustic startle response

Effects of smoking and script type on mean response amplitude in each group are presented in Fig. 2. As noted above, three separate models were estimated testing respectively for main effects, first-order interactions, and second-order interactions. Time since trauma was not significantly related to ASR during the experimental trials.

Table 3 Effect of context on negative affect and PTSD symptoms

	Non PTSD		PTSD		
Outcome	Neutral M (SE)	Trauma M (SE)	Neutral M (SE)	Trauma M (SE)	Effects
Negative affect (PANAS)	13.91 (0.62)	13.27 (0.54)	14.92 (0.74)	16.54 (0.97)	PTSD×context $F(1,225)=4.94$, $p=0.027$
PTSD B symptoms	3.13 (0.56)	4.97 (0.80)	14.60 (1.63)	16.49 (1.56)	PTSD $F(1,49)=24.85$, $p<0.0001$ Context $F(1,225)=5.04$, $p=0.0257$ PTSD×smoking F (1,225)=3.72, $p=0.0257$
PTSD C symptoms	5.35 (0.95)	6.53 (1.11)	25.38 (2.37)	27.06 (2.34)	PTSD $F(1,49)=29.78$, $p<0.0001$ Context $F(1,230)=3.75$, $p=0.054$
PTSD D symptoms	5.09 (0.79)	6.47 (0.89)	18.90 (1.79)	18.62 (1.69)	PTSD $F(1,49)=22.29$, $p<0.0001$

SE standard error

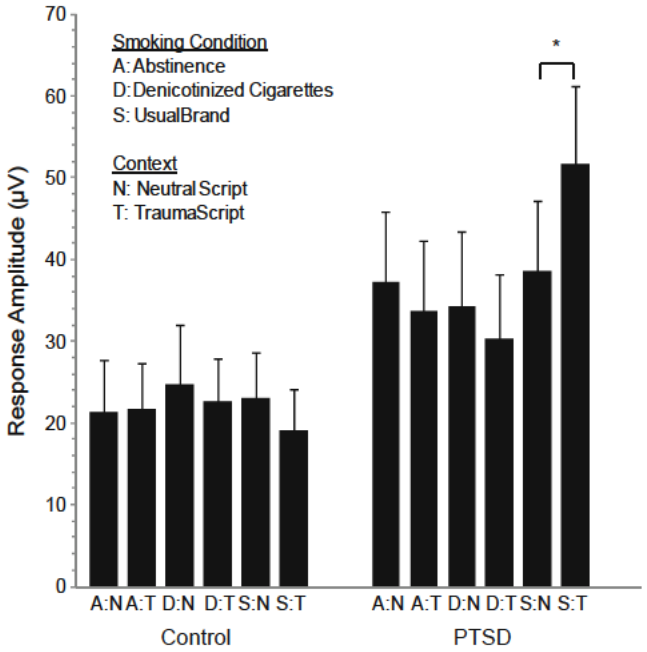


Fig. 2 Effects of smoking condition, context, and group on mean acoustic startle response amplitude. Figure shows the significant three way smoking×context×group interaction (p=0.01). *p<0.01, the specific post hoc contrast comparing the trauma script to the neutral script condition among the PTSD group following smoking a usual brand cigarette was significant

Group and context (script) were marginally significant when tested as main effects, both having a positive effect on mean response amplitude; no first-order (two-way) interactions were significant. As shown in the second-order model in Table 4, however, smoking, context, and group crossed to form a three-way interaction acting together to significantly enhance response amplitude (Wald $\chi_1^2 = 8.35$; p=0.01). That is, for participants with a PTSD diagnosis in the (usual brand) smoking condition, response amplitude was significantly higher after the presentation of a trauma script. The specific post hoc contrast evaluating response amplitude comparing the trauma script to the neutral script condition among the PTSD group following smoking a usual brand cigarette was significant ($\chi_1^2 = 6.43$; $p < 0.01$).

Discussion

Consistent with expectations, smokers with PTSD demonstrated elevated baseline ASR under conditions of overnight abstinence in comparison to smokers without PTSD. PTSD and non-PTSD participants demonstrated similar rates of habituation across the initial 10 baseline trials. In contrast, results examining the effects of group, context (neutral vs. trauma scripts), and smoking condition (abstinence vs. denicotinized cigarette vs. usual brand cigarette) during the experimental trials provided partial support for hypotheses, as discussed below.

Although previous research examining the effects of nicotine deprivation in non-psychiatric smokers on ASR has been mixed, there is some evidence that nicotine deprivation may be associated with increases in startle amplitude in comparison to startle probes initiated during a satiated state (Piasecki et al. 2002). In the context of the larger literature examining affective modulation of the ASR, startle has been consistently enhanced when participants are exposed to negative affective stimuli (Cook et al. 1992; Vrana et al. 1988). As nicotine deprivation in smokers is typically associated with increased aversive withdrawal symptoms, it was expected that nicotine deprivation would lead to greater ASR amplitude in comparison to conditions where smokers consumed a usual brand cigarette. Results indicated, however, that there was no main effect for smoking condition. Thus, on average, continued deprivation was not associated with increased ASR in comparison to those that smoked a usual brand cigarette. Further, there were no significant two-way interactions between smoking condition and group. These results are consistent with findings reported by Cinciripini and colleagues (Cinciripini et al. 2006) who reported that nicotine deprivation was associated with suppressed startle in the presence of cigarette cues, presumably because of the activation of an appetitive system, but failed to enhance startle responding to negative cues.

While there was no support for the hypothesis that nicotine deprivation would be associated with increased startle, results were consistent with the hypothesized PTSD×context interactions on measures of negative affect and startle magnitude. Ratings of negative affect after the context manipulation indicated that the trauma scripts were associated with higher levels of negative affect compared to the neutral script conditions, but only among those smokers with PTSD. Trauma scripts were also associated with an increase in PTSD re-experiencing symptoms (DSM-IV Cluster B). Further, results demonstrated a group×context×smoking interaction where trauma scripts appeared to potentiate the ASR in comparison to a neutral script, but only among PTSD participants after they had smoked a usual brand cigarette.

Consistent with many previous investigations examining affective modulation of startle in PTSD (e.g., Hamm et al. 1997; Vrana et al. 1988), the current study employed a series of habituation trials before the experimental trials. In general, startle amplitude during the experimental trials was lower than that observed during the 10 baseline trials (see Figs. 1 and 2). Although the magnitude of startle responses is expected to decrease over time, valence effects associated with affective modulation of the startle response (e.g., negative>neutral) typically remain intact (Cinciripini

Table 4 Parameter estimates of GEE analysis examining ASR response in the experimental phase

Parameter	Estimate	SE	95% confidence interval		Wald statistics for type III GEE analysis		
				+	*df*	χ_1^2	*p*
Intercept	1.924	0.379	2.666	1.182			
Premean (log)	0.698	0.055	0.591	0.805	1	163.92	<0.0001
Months since trauma	0.000	0.001	0.001	0.002		0.42	0.519
Group (PTSD vs. control)	0.420	0.378	0.320	1.160	1	0.53	0.768
Smoking					2	3.15	0.076
Usual brand	0.199	0.290	0.369	0.766			
Denicotinized	0.117	0.314	0.499	0.733			
Abstinence	0	0	0	0			
Context (trauma vs. neutral)	0.686	0.290	0.117	1.255	1	5.14	0.077
Group×smoking					2	1.79	0.18
Group×usual brand	0.336	0.393	1.107	0.435			
Group×denicotinized	0.030	0.511	0.972	1.032			
Group×abstinence	0	0	0	0			
Context×group	0.621	0.442	1.488	0.245	1	1.98	0.37
Context×smoking					2	0.14	0.71
Context×usual brand	0.828	0.381	1.576	0.081			
Context×denicotinize d	0.460	0.369	1.183	0.262			
Context×abstinence	0	0	0	0			
Context×group×smoking					2	8.35	0.015
Context×group×usual brand	1.324	0.524	0.297	2.351			
Context×group×denicotinized	0.311	0.705	1.070	1.692			
Context×group×abstinence	0	0	0	0			

et al. 2006). Current study results provide evidence that idiopathic trauma scripts were associated with increased negative affect among smokers with PTSD. Idiopathic trauma scripts also resulted in increased ASR among smokers with PTSD, but only after smoking a usual brand cigarette. In this three-way interaction, ASR levels approached the higher level demonstrated in the first few trials of the baseline habituation trials among PTSD participants. While this finding might suggest that nicotine in combination with a threatening trauma context may synergistically potentiate startle, it may also suggest that individuals with PTSD in a threatening context display a relative deficit in long-term habituation of startle after smoking. While either interpretation would be a significant finding, further research is needed to explore whether smoking a regular brand cigarette or trauma-related stimuli potentiate startle among PTSD smokers.

Our previous work has suggested that individuals with PTSD who are regular smokers and who are exposed to a trauma-related context report significant reductions in negative affect and PTSD symptoms following smoking (Beckham et al. 2007). Results from the current study, however, suggest that smoking may actually potentiate or maintain a maladaptive response, i.e., exaggerated acoustic startle. It is possible that smoking may serve to reduce negative affect in some contexts while serving to maintain other PTSD symptoms, i.e., startle. Consistent with this idea, many previous studies have identified that although smokers report that smoking a cigarette is relaxing, smoking appears to increase physiological arousal including raising basal levels of stress hormones, heart rate, and blood pressure (Kassel et al. 2003).

Still, it is important to note that the nicotine manipulation in the current study preceded the affect-induction task. In our previous work (Beckham et al. 2007), we examined the effects of smoking and nicotine after exposure to a trauma-related context. There has been no research which has examined whether smoking before exposure to a trauma-related stimulus may buffer or potentiate PTSD symptoms and negative affect among PTSD smokers. It is possible that the effects of nicotine might have been different if the nicotine manipulation occurred after affect induction.

It is also possible that nicotine exposure itself may have affected the affect induction in the current study. Because smoking occurred before the exposure to the scripts, nicotine may have enhanced engagement in (minimized avoidance of) the imagery and have changed the depth or

nature of the affect induction in some other way. The possibility that nicotine manipulation prior to affect induction may have altered that nature of the imagery stimulus induced by the trauma script is supported in part by the observation that during the nicotine-deprived conditions, the PTSD group exhibited relatively smaller (not the expected larger) ASRs during the trauma imagery. That is, when nicotine-deprived, the trauma script-associated ASRs in the PTSD group tended to be less than the neutral script ASRs (see Fig. 2). Results examining affect and PTSD symptoms, however, provide less support for this possibility. Examining affect and PTSD symptoms, there were no detected significant effects for smoking condition with one exception among PTSD participants. For PTSD re-experiencing symptoms, smoking (mean (M) = 17.36, standard deviation (SD) = 2.13) and abstinence (M = 16.61, SD = 1.91) were associated with increased symptoms while smoking denicotinized cigarettes (M=12.83, SD = 1.79) was associated with relatively lower PTSD re-experiencing symptoms. While this pattern of results is not completely consistent with the idea that nicotine may have enhanced engagement to the trauma script and altered the imagery stimulus, the possibility that individuals with PTSD did not experience comparable affective stimuli across the different smoking conditions cannot be ruled out.

It is also possible that the effects of nicotine on negative affect are moderated by potential contextual effects of trauma stimuli (e.g., internally driven versus externally driven). Gilbert (1995) has proposed a model of the effects of nicotine on attention and affect that suggests that "internally driven processes are more influenced by nicotine than are externally driven processes resulting from potent (unconditioned and high emotion elicitation potential) external stimuli," (p. 195). This model would suggest that nicotine does not reduce negative affect when the stressor is proximal and intense, as it was in the current study. That is, nicotine does not reduce negative affect when the stressor is externally driven. In the current study, the stressor was externally driven by explicit experimental demands to think about the trauma. Thus, future research might assess whether or not nicotine reduces ASR when the trauma stimuli are less externally (more internally) driven or might assess the effects of nicotine on recovery from trauma induction as assessed by ASR and other indices.

Unlike several studies that have failed to find baseline differences in startle responding between PTSD and non-PTSD groups (for reviews see, Grillon and Baas 2003; Pole 2007), there was a large effect for baseline startle observed in this relatively small sample of PTSD and non-PTSD smokers. This effect was observed under conditions of overnight abstinence from smoking. It is unclear whether satiated smokers with and without PTSD would display similar differences in baseline startle magnitude. Currently, there are no studies that have compared baseline startle responding between deprived and satiated smokers with and without PTSD. Similarly, no studies have examined whether smokers with PTSD exhibit increased startle compared to non-smokers with PTSD. These are potentially helpful questions for future research. Although results from the current study failed to detect a main effect for nicotine deprivation after a series of habituation trials, more research is needed to examine whether this is a function of possible basement effects in ASR following habituation.

The majority of previous work examining affective modulation of startle has relied on a picture-presentation paradigm (e.g., Vrana et al. 1988). The current study aimed to extend this work using a script-driven imagery paradigm (Pitman et al. 1987), which has previously distinguished PTSD from non-PTSD groups (Orr et al. 2003). The script-driven imagery paradigm has the advantage of ensuring close correspondence to idiopathic trauma exposure details; however, a weakness of this approach is that it is difficult to assess whether trauma scripts are equally aversive across PTSD and non-PTSD groups. Unlike a picture-presentation paradigm where reactivity to a standardized set of pictures is easily assessed and pictures can be chosen such that they are aversive to both groups, results in the current study suggest that the idiopathic trauma-related imagery among non-PTSD participants did not result in higher negative affect in comparison to neutral scripts. As a result, it is not surprising that there was no evidence of affective modulation among the control group, as the trauma scripts did not appear to have a significant effect on negative affective states.

Limitations of this study include the small sample of smokers, which limited the number of potential covariates included in statistical models. Further, results may not generalize all PTSD smokers. PTSD smokers in this sample had a significantly higher proportion of lifetime depression than non-PTSD smokers. Given that negative emotional symptoms are core aspects of PTSD, attempting to remove variance accounted for by depression or negative affect symptoms from PTSD would omit a defining feature of the construct and might obscure the test of study hypotheses (Feldner et al. 2008). Still, it is unknown to what extent the startle results are specific to PTSD given the high rate of comorbidity between PTSD and other psychiatric disorders. Although there was no evidence that gender was associated with ASR, groups were also unbalanced on gender. Participants were instructed to maintain their usual caffeine intake and thus were not caffeine-deprived. Future studies would be strengthened by matching on gender, history of depression, caffeine intake, and trauma history.

Despite these limitations, the current study is the first to compare ASR between smokers with and without PTSD.

Individuals with PTSD who were regular smokers demonstrated higher baseline ASR under conditions of overnight abstinence than smokers without PTSD. Results demonstrated that a script-driven imagery paradigm using idiopathic trauma scripts is a feasible method to manipulate negative affect and PTSD symptoms for the measurement of ASR for those with PTSD. Results from the three-way interaction between group, context, and smoking suggest that nicotine may potentiate or alternatively inhibit habituation in PTSD smokers in a trauma-related context. Thus, nicotine appears to potentiate or maintain a maladaptive response, acoustic startle. It would be interesting in future studies to evaluate whether smoking cessation is associated with improved startle function among those with PTSD.

Acknowledgments The authors thank the participants for their assistance in this research. This material is based upon work supported in part by R21DA019704, R21CA12896, 2K24DA016388, and the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Clinical Sciences Research and Development, and Health Services Research and Development.

Conflict of interest The views expressed in this article are those of the authors and do not necessarily represent the views of the Department of Veterans Affairs, the National Institutes of Health, or the United States government. The authors have no competing interests.

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Experimental and Clinical Psychopharmacology
2011, Vol 19, No 2, 105–115

© 2011 American Psychological Association
1064-1297/11/$12 00 DOI: 10 1037/a0022937

The Influence of Nicotine Dose and Nicotine Dose Expectancy on the Cognitive and Subjective Effects of Cigarette Smoking

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This study investigated the independent and interactive effects of nicotine dose and nicotine dose expectancy on smoking outcomes using a 2 (given nicotine vs. placebo) × 2 (told nicotine vs. placebo) Balanced Placebo Design (BPD). Smokers (N = 148) completed the Rapid Visual Information Processing Task (RVIP) and measures of smoking urge, mood, and cigarette ratings (e.g., satisfying) after smoking a nicotine or placebo cigarette crossed with instructions that the cigarette contained either nicotine or no nicotine. Nicotine cigarettes (0.6 mg nicotine) produced better sustained attention performance than placebos as indicated by RVIP reaction time, hits, and sensitivity (A′). Nicotine cigarettes also produced better mood and greater rewarding subjective effects of the cigarettes on 11 of 11 dimensions compared to placebos. Nicotine instructions resulted in fewer RVIP false alarms, better mood, and greater rewarding subjective effects of the cigarettes on 9 of 11 dimensions compared to placebo instructions. Nicotine dose by nicotine dose expectancy interactions were also observed for urge and tension-anxiety, such that the dose expectancy manipulation produced differential effects only among those who smoked placebo cigarettes. In contrast a significant interaction for self-reported vigor-activity demonstrated that the dose expectancy manipulation produced effects only among those who smoked nicotine cigarettes. This study provides additional evidence that nicotine improves cognitive performance, and provides initial evidence that denicotinized cigarettes smoked under the guise that they contain nicotine influence cognitive performance, albeit with less robust effects than nicotine. These data may inform the development of expectancy-based interventions for tobacco dependence.

Keywords: smoking, expectancies, placebo, nicotine, cognitive performance

In addition to nicotine, there is evidence that nonpharmacological factors such as expectancy play a role in the immediate rewarding effects of cigarette smoking. According to expectancy theory, a smoker will experience urge reduction in response to smoking a placebo cigarette, if the smoker has the *stimulus (or dose) expectancy* that he or she is smoking an active nicotine cigarette and has the *response expectancy* that nicotine reduces urges to smoke (Kirsch & Lynn, 1999; Perkins, Sayette, Conklin, & Caggiula, 2003). Expectancy theory proposes that response expectancies produce direct self-confirming automatic responses (e.g., negative affect reduction) to a situation (e.g., treatment, drug, or placebo) and can explain placebo and nocebo effects (Kirsch & Lynn, 1999). Conditioning processes have also been offered as an explanation for placebo effects.[1] For example, several studies have demonstrated that denicotinized cigarettes smoked under double-blind conditions alleviate smoking craving and withdrawal symptoms (e.g., Buchhalter, Acosta, Evans, Breland, & Eissenberg, 2005; Donny, Houtsmuller, & Stitzer, 2007) and function as a reinforcer (Shahan, Bickel, Madden, Badger, 1999). The sensorimotor aspects of smoking (e.g., sensations of smoke in one's throat) presumably become conditioned stimuli due to their previous associations with nicotine, thereby producing rewarding effects in the absence of nicotine (Rose, 2006). However, research has shown that denicotinized cigarettes smoked under different dose expectancy sets produce different effects, suggesting an important role of expectancy.

Controlled research studies across a range of drugs, including cigarette smoking and nicotine replacement products provide evidence that directly manipulating dose ex-

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This research was funded by Grant RO3 DA18709 from the National Institute on Drug Abuse.

We thank Peter G. Kardel for his assistance recruiting and running participants, and managing the data set. We thank David Gilbert and F. Joseph McClernon for consultation on the RVIP task.

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[1] The empirical evidence for expectancy theory and conditioning as mechanisms of placebo effects is reviewed by Stewart-Williams and Podd (2004).

pectancies and/or response expectancies (i.e., manipulating the expected effects of the drug) can influence the magnitude of responses to placebos and active drugs (e.g., Fillmore & Vogel-Sprott, 1996; Fillmore, Mulvihill & Vogel-Sprott, 1994; Fucito & Juliano, 2007; Harrell & Juliano, 2009; Metrik et al., 2009; Perkins et al., 2006; Perkins et al., 2009). Understanding the direct role that nonpharmacological factors such as expectancy play in the full range of effects of cigarette smoking may help us to better understand the persistence of smoking behavior and develop innovative expectancy-based treatment strategies (Copeland & Brandon, 2000).

An important motivation for smoking that has yet to be adequately addressed in smoking expectancy research is the enhancement of cognitive performance. It has been suggested that the effects of smoking on cognition, mood and arousal are likely connected in systematic ways and are all outcomes that smokers are seeking from nicotine (Waters & Sutton, 2000). Smoking for cognitive enhancement is reported as a common motive for smoking (Gilbert et al., 2000), and a large body of experimental research has confirmed that smoking and/or nicotine improves performance on cognitive processes including but not limited to sustained attention, alerting attention, orienting attention, episodic memory, and working memory (Heishman, Kleykamp, & Singleton, 2010; Koelega, 1993; Prichard & Robinson, 1998). Improvements in sustained attention (or vigilance) observed after smoking appear to be particularly robust (Koelega, 1993).

A recent study evaluated vigilance performance, memory, and subjective effects in 5-hr abstinent smokers before and after smoking a nicotine or denicotinized cigarette under double-blind conditions (Kelemen, 2008). After smoking participants were asked to guess which type of cigarette they smoked (resulting in a post hoc quasi independent variable to assess *dose expectancy*) and rate the effect that the cigarette has (or had) on his or her performance on the cognitive tasks (*response expectancy*). The authors concluded that dose expectancy partially mediated the relationship between nicotine and subjective effects, and that response expectancies assessed after participants completed the cognitive tasks, but not before, were associated with actual performance in some cases. However, this retrospective design prohibits making causal interpretations about the role of expectancies in smoking outcomes. It is quite possible that participants who had the most salient pharmacological effects of nicotine simply were more likely to guess correctly that they smoked a nicotine cigarette as well as report that nicotine had a greater effect on their performance.

The present study utilized the Balanced Placebo Design (BPD) to examine the causal roles of nicotine dose expectancies and nicotine dose in the cognitive and subjective effects of cigarette smoking. The BPD is a 2×2 factorial design (Rohsenow & Marlatt, 1981) in which the experimenter manipulates both the content of the drug (e.g., nicotine vs. placebo cigarette) and the information provided to participants about drug content, or *dose expectancy* (e.g., told nicotine or placebo cigarette). In the case of smoking the four conditions are: (a) Given Nicotine/Told Nicotine, (b) Given Nicotine/Told Placebo, (c) Given Placebo/Told Nicotine, and (d) Given Placebo/Told Placebo (Perkins et al., 2003). Less than a handful of published studies to date have used the BPD with cigarette smoking, with denicotinized cigarettes functioning as placebos (Juliano & Brandon, 2002; Kelemen & Kaighobadi, 2007; Perkins et al., 2008; Perkins et al., 2004). As a whole these studies provide evidence that the belief that one is smoking a nicotine cigarette, independent of actual nicotine ingestion, leads to greater reports of smoking liking and/or satisfaction (Kelemen & Kaighobadi, 2007; Perkins et al., 2004; Perkins et al., 2008), reduced smoking urges and/or cravings (Kelemen & Kaighobadi, 2007; Perkins et al., 2008), greater rewarding subjective effects of smoking (Kelemen & Kaighobadi, 2007), and decreased latency to smoke (Perkins et al., 2008). Not surprisingly, smoking BPD studies have also shown that relative to placebos, nicotine cigarettes better alleviate smoking urges or cravings (Juliano & Brandon, 2002), withdrawal symptoms (Juliano & Brandon, 2002; Perkins et al., 2008), and negative affect (Juliano & Brandon, 2008; Kelemen & Kaighobadi, 2007) as well as produce greater reports of smoking satisfaction (Juliano & Brandon, 2002: Perkins et al., 2004; Perkins et al., 2008) and other rewarding effects of smoking (Juliano & Brandon, 2002; Kelemen & Kaighobadi, 2007; Perkins et al., 2008). At times smoking BPD studies have revealed significant interactions between nicotine pharmacology and nicotine dose expectancies such that nicotine dose expectancy manipulations have had greater effects on subjective (e.g., urge) or behavioral responses (i.e., work for cigarette puffs) when placebo or low nicotine cigarettes are smoked compared to nicotine cigarettes (Juliano & Brandon, 2002; Perkins et al., 2004). Nicotine dose and nicotine dose expectancy usually show additive effects with the magnitude of positive responses typically largest in the given nicotine/told nicotine condition and smallest in the given placebo/told placebo conditions. Some BPD studies have also identified moderators of reactions to the dose expectancy manipulations including baseline smoking outcome expectancies (Juliano & Brandon, 2002), mood state (Perkins et al., 2008), and gender (Perkins et al., 2004).

To expand upon this prior work, the present study used the BPD to evaluate the independent and interactive effects of nicotine pharmacology and nicotine dose expectancy on smokers' responses to the Rapid Visual Information Processing (RVIP) task, a commonly used test of sustained attention. In addition, subjective effects were assessed with measures of smoking urge, mood, and rewarding effects of the cigarette (e.g., satisfaction); each of which has been shown to play a role in smoking behavior. Main effects for both nicotine and nicotine dose expectancy were hypothesized such that participants who were either told nicotine or given nicotine would show greater sustained attention performance and positive subjective effects in response to smoking compared to those told placebo or given placebo. We were also interested in identifying possible interactions between nicotine dose and nicotine dose expectancies.

Method

Participants

One hundred and fifty-three smokers (87 males and 66 females) were recruited from American University and the local Washington, D.C. community and 148 participants (82 males and 66 females) completed the two day protocol. Inclusion criteria were as follows: at least 18 years of age, a smoking history of at least 10 cigarettes per day for a minimum of one year, and no chronic smoking-related medical condition or psychiatric condition that would interfere with study participation. Participants (mean age = 29.65, *SD* = 12.24) smoked a mean of 13.56 (*SD* = 5.60) cigarettes per day for a mean of 10.59 years (*SD* = 10.30). The mean Fagerström Test of Nicotine Dependence score was 3.76 (*SD* = 2.11). Most of the participants identified themselves as either Caucasian (55%) or African American (34%). Fewer than half of the participants were full-time students. Participant characteristics across the four conditions are shown in Table 1.

Measures and Materials

Breath carbon monoxide (CO). Carbon monoxide levels were measured in parts per million from breath samples using a Bedfont Micro III Smokerlyzer (Kent, U.K.). Breath CO samples were taken to encourage compliance with instructions to abstain from smoking for three hours prior to arrival. Presmoking to postsmoking changes in CO level also provided a rough measure of the amount of smoke inhaled during the experimental manipulation.

Demographics/smoking history questionnaire. A 15-item questionnaire was developed for this study to assess demographic information and smoking history. It included the Fagerström Test of Nicotine Dependence (Heatherton, Kozlowski, Frecker, & Fagerström, 1991), which is a widely used six item measure of nicotine dependence.

Smoking consequences questionnaire-adult. This 55-item questionnaire (SCQ-A; Copeland, Brandon, & Quinn, 1995) assesses smoking outcome expectancies a scale from *0 = Completely unlikely* to *9 = Completely likely*. The items cluster into the following 10 factors: (a) negative affect reduction, (b) stimulation/state enhancement, (c) health risk, (d) taste/sensorimotor stimulation, (e) social facilitation, (f) weight control, (g) craving/addiction, (h) negative physical feelings, (i) boredom reduction, and (j) negative social impression. It is reliable (alpha coefficients ranged from 0.83 to 0.96) and associated with smoking status, level of dependence, and smoking treatment outcome.

Urge rating scale. This three item self-report measure assessed participants' cravings, wants, and desires to smoke on scale from *1 = strongly disagree* to *7 = strongly agree*. These three items have demonstrated adequate reliability and validity in assessing smoking urge (Kozlowski, Pillitteri, Sweeney, Whitfield & Graham, 1996). Cronbach's alpha in the present study averaged 0.95.

Profile of mood states-short form (POMS-SF). The short form of the POMS (McNair, Lorr, & Droppelman, 1971) is a 30-item adjective checklist that assessed total mood disturbance and mood specific problems using six subscales: (a) tension-anxiety, (b) depression-dejection, (c) anger-hostility, (d) vigor-activity, (e) fatigue-inertia, and (f) confusion-bewilderment. Participants rated the items on a 5-point scale ranging from *0 = not at all* to *4 = extremely*. The short form has good psychometric properties and is highly correlated with the full 65 item scale. Cronbach's alpha for the individual factors was good (>.80) on all factors except for confusion-bewilderment (not analyzed), and for total mood disturbance (including confusion-bewilderment items) was excellent (*M* = 0.92).

Cigarette evaluation scale. This 14-item questionnaire (Rose, Behm, & Westman, 2001) assessed participants' immediate reactions to the experimental cigarettes using a 7-point scale from *1 = not at all* to *7 = extremely*. In reference to the cigarette that participants had just smoked, they were asked questions such as: Was it satisfying?, Did it taste good?, Did you enjoy the sensations of smoke in your throat and chest? In addition to the 10 items described by Rose et al. (2001) the following four items were also included: (a) Did it immediately reduce your cravings for cigarettes?, (b) Did it taste different than your usual brand?, (c) Did it make you feel more alert? and (d) Did it make you feel less anxious?

Rapid visual information processing (RVIP). The RVIP is a test of sustained attention or vigilance. This task has been used in many studies of drug effects, and has been shown to be especially sensitive to the effects of smoking and nicotine (Koelega, 1993; Prichard & Robinson, 1998). Participants viewed a series of single digits presented on the computer screen at a rate of 100 digits per minute for 12 minutes. Participants were told to press a mouse button as quickly as possible whenever they detected three consecu-

Table 1
Baseline Values for Demographic Variables and Dependent Measures

Variable[1]	Mean (*SD*)
Age (years)	29.65 (12.23)
Cigarettes per Day	13.56 (5.60)
Years Smoked	10.59 (10.30)
FTND	3.76 (2.11)
Carbon Monoxide	7.72 (4.58)
Self Reported Urge	5.49 (1.71)
POMS Depression	2.18 (3.33)
POMS Anger	2.86 (4.20)
POMS Tension	3.94 (3.72)
POMS Fatigue	4.96 (4.67)
POMS Vigor	6.91 (4.67)
POMS Total Mood Disturbance	31.32 (17.28)
RVIP Reaction Time	516.17 (124.62)
RVIP Hits	35.33 (16.45)
RVIP False Alarms	17.99 (35.55)
RVIP Sensitivity	0.79 (0.19)

Note. [1] A series of 2 × 2 ANOVAS indicated that there were no significant differences between groups at baseline on any variables (all *p*'s > .1). FTND = Fagerström Test of Nicotine Dependence; POMS = Profile of Mood States-Short Form; RVIP = Rapid Visual Information Processing Task.

tive odd or three consecutive even numbers (96 potential hits). To increase motivation to perform well on the RVIP task and improve sensitivity, on the experimental day participants were informed that they would earn 2.5 cents for each correct detection (hit) and lose 2.5 cents for each false alarm. Response targets appeared eight times per minute with 8 to 36 digits appearing between each target. Reaction time and responses (hits and false alarms) were recorded by the computer. Responses that occurred 100 ms to 1,500 ms after the target were scored as hits. Sensitivity, which takes into account both the number of hits and false alarms was computed as $A' = .5 + [(hr\text{-}far) + (hr\text{-}far)^2]/4^*hr^*(1\text{-}far)]$. Response bias was computed as $B'[(h\text{-}h)^2\text{-}(f\text{-}f)^2]/[(h\text{-}h)^2 + (f\text{-}f)^2]$ (Saghal, 1987). The RVIP was administered using DirectRT software (Empirisoft Research Software, New York. Available from http://www.empirisoft.com/). The RVIP was administered during a practice session, and then on the experimental day both before and after smoking the experimental cigarette as has been done in previous smoking studies (see Prichard & Robinson, 1998).

Experiment evaluation form. This measure was developed primarily to evaluate the credibility of the dose expectancy manipulation. Because of the deception involved in the BPD methodology, it is important to assess if participants believe what they are told about the nicotine dose of the cigarette. At the end of the study, participants were asked to indicate the type of cigarette they smoked by endorsing one of five choices: (a) definitely placebo, (b) probably placebo, (c) I don't know, (d) probably nicotine, or (e) definitely nicotine. Those who were told nicotine but who endorsed either of the placebo choices and those who were told placebo but endorsed either of the nicotine choices were coded as "nonbelievers" for later analyses.

Experimental cigarettes. The experimental cigarettes were marketed under the trade name *Quest* (Vector Tobacco, Timberlake, NC). The nicotine cigarette contained 0.6 mg of nicotine and 10 mg of tar (*Quest* 1) and the placebo cigarette contained no more than 0.05 mg nicotine and 10 mg of tar (*Quest* 3). Although the placebo cigarettes contain a small amount of nicotine, the dose absorbed is unlikely to produce pharmacological effects (Pickworth, Fant, Nelson, Rohrer, & Henningfield, 1999). Participants were given either a menthol cigarette (36%) or nonmenthol cigarette (64%) depending on their usual smoking preferences.

Procedure

Participants who responded to the advertisements were informed that this was a study investigating "immediate reactions to smoking a brand of commercially available cigarettes." Those who met eligibility requirements were scheduled to attend a baseline visit during which participants gave informed consent, provided a CO breath sample, completed baseline measures (Demographics, SCQ-A, POMS-SF, and Urge[2]) and completed a 12-min practice trial of the RVIP. No smoking took place during the baseline session.

A second laboratory visit was scheduled within the next two days and participants were instructed to abstain from smoking for three hours prior to their appointment time. Upon arrival participants were taken to an experimental room where they provided a CO breath sample and were queried about compliance with the abstinence requirement. Those who reported smoking within the past three hours were rescheduled to complete the experiment on another day ($n = 5$). After this point all measures, tasks, and instructions were delivered via computer. First, participants completed measures of smoking urge, mood (POMS-SF), and the 12 minute RVIP task. Next, participants were instructed by the computer to remove a cigarette from a box that was located on the desk. The cigarette contained either 0.6 mg nicotine or 0.05 mg nicotine depending on participant assignment and was placed in the box before the participant arrived. The computer screen presented one of two scripts with corresponding voiceover depending on whether the participant was assigned to the told nicotine or told placebo condition. Thus, there were four experimental conditions: (a) Given Nicotine/Told Nicotine ($n = 37$), (b) Given Nicotine/Told Placebo ($n = 40$); (c) Given Placebo/Told Nicotine ($n = 36$), and (d) Given Placebo/Told Placebo ($n = 35$).

> **Told nicotine instructions.** In this portion of the experiment, you are asked to smoke a cigarette and then rate the cigarette. The cigarette you will smoke is a normal tobacco cigarette than contains a standard amount of nicotine. Please remove the cigarette, lighter, and ashtray from the box that is located next to the computer. Please light and smoke the cigarette as you would normally smoke.
>
> **Told placebo instructions.** In this portion of the experiment, you are asked to smoke a cigarette and then rate the cigarette. The cigarette you will smoke is a normal tobacco cigarette except that it is a placebo; it contains no nicotine. Please remove the cigarette, lighter, and ashtray from the box that is located next to the computer. Please light and smoke the cigarette as you would normally smoke.

Having the dose expectancy manipulation delivered via computer allowed experimenters to be blind to both the nicotine content of the cigarette and the dose expectancy manipulation. Participants were observed smoking through a one-way mirror and experimenters recorded the smoking duration and number of puffs taken. Participants were instructed to press the left mouse button key when they were finished smoking, which began the computerized administration of postsmoking measures including cigarette ratings (CES), urge, mood (POMS-SF) and the 12 minute RVIP task.

The experimenter then returned to the room to inform participants that the experiment was over and to administer the experiment evaluation form. Another CO sample was taken and then participants were debriefed about the purpose of the experiment (to evaluate reactions to smoking

[2] Participants also completed a measure of expectancies for nicotine that was not used in the present analyses as it has not yet been validated.

cigarettes with different levels of nicotine), but not about the nicotine content of the experimental cigarette. Information about the experimental cigarette was provided to participants at a later date by the primary investigator. Participants were paid $20 plus any additional compensation they earned based on their RVIP performance (up to $4.80). After the participant left the laboratory, the experimenter weighed the cigarette remains.

Results

Data Analytic Strategy

A series of 2 (nicotine dose) × 2 (dose expectancy) analyses of variance (ANOVAs) were conducted on measures that were collected at only one time point (i.e., CES, measures of smoking behavior) and to evaluate baseline equivalence on all measures collected on the practice day. A series of 2 (nicotine dose) × 2 (dose expectancy) analyses of covariance (ANCOVA's) were conducted on measures that were collected before and after smoking (e.g., CO, urge, mood, and RVIP indices) with the presmoking score entered as the covariate and postsmoking score entered as the dependent variable. Significant interactions were followed up with simple comparisons of the dose expectancy manipulation in the given nicotine groups (Given Nicotine/Told Nicotine vs. Given Nicotine/Told Placebo) and the given placebo groups (Given Placebo/Told Nicotine vs. Given Placebo/Told Placebo). All analyses were conducted at first without regard to gender and then repeated including gender as an independent variable (see Perkins et al., 2006). There were no effects of gender on any of the dependent measures so all analyses are reported with men and women combined.

Baseline Data

There were no differences between groups at baseline on expired air CO, age, race, smoking rate, years smoked, nicotine dependence (FTND), smoking outcome expectancies (SCQ-A), or any of the dependent measures (all p's $>.10$). Table 1 shows the means and standard deviations for the entire sample.

Manipulation Checks

Dose expectancy manipulation. To test the credibility of the dose expectancy manipulation participants were asked to respond to a question that asked what type of cigarette they smoked. Eighty-two percent of participants ($n = 122$) reported smoking a cigarette consistent with what they were told. Not surprisingly, the highest rates of disbelief were in the conditions in which participants were deceived with 15% (6/40) of those in the given nicotine/told placebo group and 36.11% (13 of 36) of those in the given placebo/told nicotine group not believing the manipulation. In contrast 10.81% (4/37) of those in the given and told nicotine group and 8.57% (3/35) in the given and told placebo group reported smoking a cigarette type different from what they were told. All analyses were conducted using both the subsample of 122 participants who provided reports consistent with the nicotine dose expectancy manipulation and the full sample of 148 participants. There were no differences in findings among the two types of analyses. Thus, we present data using the full sample of participants to maintain roughly equivalent sample sizes and utilize all collected data.

Cigarette manipulation. The next set of analyses was performed to examine how participants smoked the experimental cigarettes. As shown in Table 2, there was a main effect of nicotine dose on smoking duration with participants in the given nicotine groups smoking longer than those in the given placebo groups, $M = 216.37$s versus $M = 180.77$s, $F(1, 143) = 13.36$, $p < .001$, $\eta^2 = 0.09$. There was a significant dose expectancy by nicotine dose interaction for total number of cigarette puffs, $F(1, 143) = 13.34$, $p < .001$, $\eta^2 = 0.09$. Simple comparisons revealed that among participants given nicotine those told nicotine took a greater number of puffs than those told placebo $F(1, 75) = 8.45$, $p = .005$, but among those given placebo, those told nicotine took fewer puffs than those told placebo, $F(1, 68) = 5.20$, $p = .026$. There were no significant group differences on the weights of postsmoking cigarette remains ($M = .49$, $SD = .09$) or CO boost ($M = 3.52$, $SD = 2.41$).

Dependent Variables

Cognitive performance. As shown in Figure 1, there was a main effect of nicotine on RVIP reaction time with those given nicotine exhibiting shorter reaction times to targets than those given placebo, $F(1, 141) = 4.28$, $p = .04$, $\eta 2 = 0.030$. Participants given nicotine also had a greater number of hits ($M = 39.40$, SE 1.50) than those given placebo ($M = 34.63$, SE 1.58), $F(1, 141) = 9.53$, $p = .002$, $\eta^{2\ =}\ 0.067$, and showed greater sensitivity on the task, $F(1, 141) = 4.26$, $p = .041$, $\eta^2 = 0.030$. As shown in Figure 2, there was a main effect of dose expectancy on the number of false alarms such that those told nicotine had fewer false alarms than those told placebo, $F(1, 141) = 6.94$, $p = .009$, $\eta^2 = 0.049$. There were no effects of dose expectancy or nicotine dose on response bias, and no dose expectancy by nicotine dose interactions.

Smoking urge. For smoking urge, there was a significant nicotine dose by dose expectancy interaction, $F(1, 143) = 5.47$, $p = .020$, $\eta^2 = 0.04$. As depicted in Figure 3, simple effects tests revealed no dose expectancy effect among participants given nicotine, $F(1, 74) = 2.22$, $p = .141$, but a significant dose expectancy effect among participants given placebo, $F(1, 68) = 32.31$, $p < .0001$, with those told nicotine reporting lower smoking urge than those told placebo.

Mood. There were main effects for nicotine, $F(1, 143) = 16.35$, $p < .01$, $\eta^2 = 0.12$ and dose expectancy, $F(1, 143) = 7.18$, $p < .01$, $\eta^2 = 0.05$ for total mood disturbance, with those given nicotine showing less mood disturbance than those given placebo and those told nicotine showing less mood disturbance than those told placebo. Similarly, main effects for nicotine dose and dose expectancy were found for the depression-dejection and anger-hostility fac-

Table 2
Measures of Smoking Exposure

	Condition (Given/Told)				Effect			
	Nicotine/ Nicotine	Nicotine/ Placebo	Placebo/ Nicotine	Placebo/ Placebo	Main effect nicotine	Main effect dose expectancy	Nicotine X dose expectancy
	Mean[a] *(SEM)*	Mean *(SEM)*	Mean *(SEM)*	Mean *(SEM)*	F η^2	F η^2	F η^2
Smoking duration (s)	214.81 (9.69)	217.93 (9.32)	182.44 (9.83)	179.09 (10.11)	$F = 13.36^{***}$, 0.09	*ns*	*ns*
Number of puffs	13.76 (0.56)	11.28 (0.54)	9.94 (0.57)	11.56 (0.58)	$F = 9.90^{**}$, 0.07	*ns*	$F = 13.34^{**}$, 0.09
CO boost	3.43 (0.40)	3.22 (0.40)	3.41 (0.42)	4.03 (0.42)	*ns*	*ns*	*ns*
Cigarette weight (g)	0.48 (0.01)	0.48 (0.01)	0.50 (0.01)	0.49 (0.01)	*ns*	*ns*	*ns*

Note. CO = Carbon Monoxide.
$^{\dagger}p < .08$. $^{*}p < .05$. $^{**}p < .01$. $^{***}p < .001$.

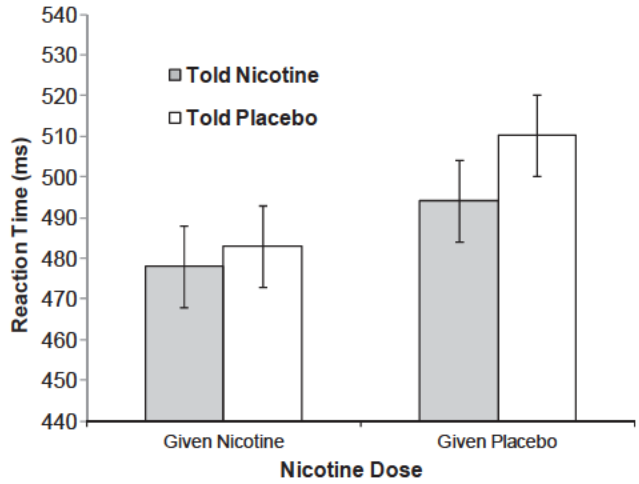


Figure 1. Reaction time (ms) to RVIP targets across the four experimental conditions showing a main effect of nicotine dose.

tors (see Table 3), and only nicotine dose on the fatigue-inertia factor. Significant interactions were observed for the tension-anxiety and vigor-activity factors with simple comparisons revealing differences between the told nicotine and told placebo conditions only in the context of placebo for tension-anxiety, $F(1, 68) = 7.29$, $p = .009$ and only in the context of nicotine for vigor-activity, $F(1, 74) = 5.32$, $p = .024$.

Subjective ratings of the cigarettes. As shown in Table 4, main effects of nicotine dose were observed for all 11 rewarding subjective ratings of the cigarette and main effects of dose expectancy were observed for 9 of 11 rewarding subjective cigarette ratings. Main effects of nicotine dose were also observed for ratings of dizzy and tastes different than usual brand. There were no interactions.

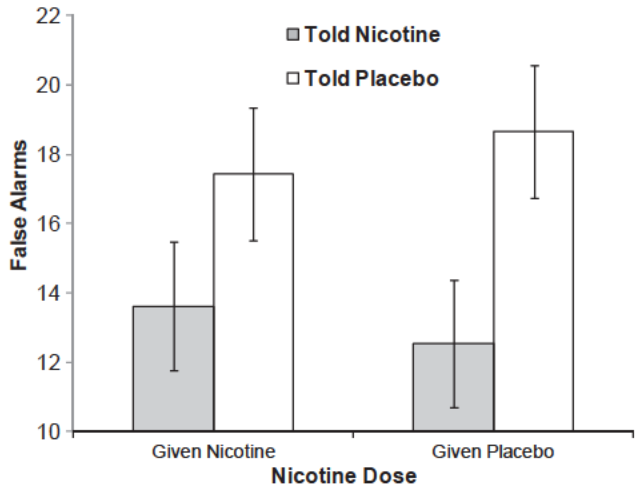


Figure 2. Number of RVIP false alarms across the four experimental conditions showing a main effect of nicotine dose expectancy.

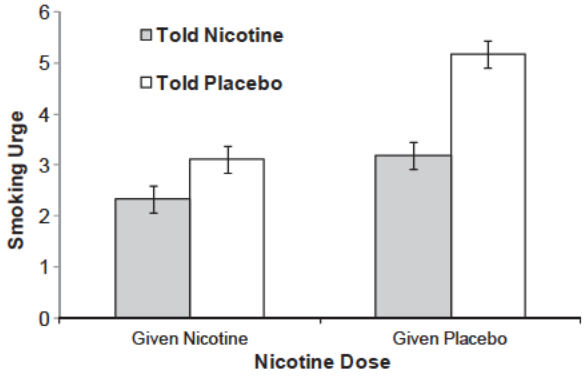


Figure 3. Mean self-reported smoking urge across the four experimental conditions. Simple effects tests following a significant nicotine dose by dose expectancy interaction revealed an effect of the nicotine dose expectancy manipulation in the context of placebo administration but not nicotine administration.

Discussion

This study is the first to evaluate the independent and interactive effects of nicotine dose and nicotine dose expectancy on cognitive performance improvements after cigarette smoking. Consistent with many previous studies, nicotine cigarettes led to better performance on a test of sustained attention (RVIP) than placebo cigarettes. Three-hour abstinent smokers who smoked nicotine cigarettes demonstrated faster reaction time, greater accuracy (hits), and greater sensitivity on the RVIP. There was no effect of nicotine on response bias. Participants who were told that the cigarette was a placebo produced a greater number of false alarms than those told nicotine, regardless of actual dose. Interestingly, the rate of false alarms is typically not influenced by nicotine administration (Koelega, 1993; Prichard & Robinson, 1998), but in this case appeared to be influenced by dose expectancy. However, the size of this effect was small and this preliminary finding should be followed up with additional research.

As far as urge to smoke, there was a nicotine dose by dose expectancy interaction. As has been found in other smoking BPD studies (Juliano & Brandon, 2002; Kelemen & Kaighobodi, 2007), being told nicotine produced significantly lower urge than being told placebo, but only in the context of smoking a placebo cigarette. The dose expectancy manipulation had little effect in the context of actual nicotine administration. This suggests that either nicotine or the belief that one is smoking a nicotine cigarette is sufficient to attenuate smoking urges, but that dose expectancies are not adding to the effect of nicotine. Perkins and colleagues (2008) found that those who were told nicotine reported lower smoking urges relative to those told placebo, but only in the context of positive mood not a negative mood. It has been suggested that stressful cognitive situations may moderate the relative contributions of nicotine and smoking somatosensory stimulation to smoking craving (Baldinger, Hasenfratz, & Battig, 1995). Thus it is possible that our findings may not generalize across different mood states.

Table 3
Covariate Adjusted Profile of Mood States Ratings Across the Experimental Conditions

	Condition (Given/Told)				Effect			
	Nicotine/ Nicotine	Nicotine/ Placebo	Placebo/ Nicotine	Placebo/ Placebo	Main effect nicotine	Main effect dose expectancy	Nicotine X dose expectancy	
	Mean[a] (*SEM*)	Mean (*SEM*)	Mean (*SEM*)	Mean (*SEM*)	F η^2	F η^2	F η^2	
POMS Depression-Dejection	1.17 (0.29)	1.49 (0.28)	1.74 (0.29)	*2.70 (0.30)*	F[b] = 9.51** 0.066	F = 4.95* 0.035	*ns*	
POMS Anger-Hostility	1.32 (0.49)	2.51 (0.48)	3.01 (0.50)	4.35 (0.51)	F = 12.75*** 0.089	F = 6.60* 0.046	*ns*	
POMS Tension-Anxiety	2.24 (0.43)	2.40 (0.42)	2.97 (0.44)	4.84 (0.44)	F = 13.47*** 0.094	F = 5.50* 0.039	F = 3.93[c]* 0.027	
POMS Fatigue	3.87 (0.46)	4.35 (0.44)	4.68 (0.47)	5.21 (0.47)	F = 3.28† 0.023	*ns*	*ns*	
POMS Vigor	7.70 (0.65)	5.14 (0.62)	5.50 (0.65)	5.58 (0.66)	*ns*	F = 3.56† 0.025	F = 4.22[d]* 0.030	
POMS Total mood disturbance	24.81 (1.66)	29.41 (1.60)	31.68 (1.69)	36.06 (1.74)	F = 16.35*** 0.115	F = 7.18** 0.051	*ns*	

Note. SEM = Standard Error of the Mean. η^2 = effect size.
[a] covariate adjusted means, baseline score as covariate. [b] df (1,143) for all analyses. [c] Effect of dose expectancy in the context of placebo administration with those told placebo reporting greater tension than those told nicotine. $F(1, 68) = 7.29, p = .009$. No effect of dose expectancy in the context of nicotine administration. [d] Effect of dose expectancy in the context of nicotine administration with those told placebo reporting less vigor than those told nicotine, $F(1, 74) = 5.32, p = .024$. No effect of dose expectancy in the context of placebo administration.
$^{\dagger} p < .08$. $^{*} p < .05$. $^{**} p < .01$. $^{***} p < .001$.

Table 4
Cigarette Ratings Across the Experimental Conditions

	Conditions (Given/Told)				Effects			
	Nicotine/ Nicotine	Nicotine/ Placebo	Placebo/ Nicotine	Placebo/ Placebo	Main effect nicotine	Main effect instructions	Nicotine X instructions
	Mean (*SEM*)	Mean (*SEM*)	Mean (*SEM*)	Mean (*SEM*)	F η^2	F η^2	F η^2
Satisfying	5.40 (0.24)	4.30 (0.23)	3.17 (0.24)	2.29 (0.25)	$F^a = 78.68^{***}$ 0.546	$F = 17.16^{***}$ 0.119	*ns*
Tastes good	4.41 (0.25)	3.70 (0.24)	2.53 (0.25)	1.94 (0.26)	$F = 52.85^{***}$ 0.367	$F = 6.66^{*}$ 0.046	*ns*
Enjoyable sensations in throat and chest	3.86 (0.26)	3.48 (0.25)	3.17 (0.26)	2.29 (0.27)	$F = 13.36^{***}$ 0.093	$F = 6.05^{**}$ 0.042	*ns*
Calming	4.46 (0.22)	3.43 (0.22)	2.89 (0.23)	1.86 (0.23)	$F = 49.05^{***}$ 0.341	$F = 21.26^{***}$ 0.148	*ns*
Less irritable	3.68 (0.25)	2.58 (0.24)	2.50 (0.25)	1.77 (0.26)	$F = 15.54^{***}$ 0.108	$F = 13.27^{***}$ 0.092	*ns*
Helped Concentrate	3.43 (0.24)	2.88 (0.23)	2.28 (0.25)	1.80 (0.25)	$F = 20.88^{***}$ 0.145	$F = 4.50^{*}$ 0.031	*ns*
Reduced craving	4.86 (0.27)	4.00 (0.26)	3.81 (0.27)	2.11 (0.28)	$F = 29.74^{***}$ 0.207	$F = 22.40^{***}$ 0.156	*ns*
Less anxious	3.78 (0.23)	2.65 (0.23)	2.75 (0.23)	1.69 (0.24)	$F = 18.97^{***}$ 0.132	$F = 22.95^{***}$ 0.159	*ns*
More alert	3.10 (0.21)	2.48 (0.20)	2.19 (0.21)	1.97 (0.21)	$F = 11.80^{***}$ 0.082	$F = 4.31^{*}$ 0.030	*ns*
More awake	3.38 (0.24)	2.75 (0.23)	2.25 (0.24)	2.00 (0.24)	$F = 15.79^{***}$ 0.110	$F = 3.45^{\dagger}$ 0.024	*ns*
Reduced hunger	2.30 (0.22)	2.45 (0.21)	1.94 (0.22)	1.34 (0.22)	$F = 11.26^{**}$ 0.078	*ns*	*ns*
Different from usual brand	4.59 (0.23)	4.85 (0.22)	6.31 (0.23)	6.49 (0.23)	$F = 54.63^{***}$ 0.379	*ns*	*ns*
Dizzy	2.73 (0.27)	2.83 (0.26)	1.83 (0.27)	1.57 (0.28)	$F = 15.72^{***}$ 0.109	*ns*	*ns*
Nauseous	1.38 (0.17)	1.58 (0.16)	1.64 (0.17)	1.94 (0.17)	$F = 3.49^{\dagger}$ 0.023	*ns*	*ns*

Note. SEM = Standard Error.
[a] df (1,144) for all analyses.
$^{\dagger}p < .08$. $^{*}p < .05$. $^{**}p < .01$. $^{***}p < .001$.

One prior smoking BPD study (Perkins et al., 2004) did not find any effects on smoking urge. However, that study differed from the others in that presmoking abstinence was very brief (only one hr) and participants took only two puffs on the experimental cigarette prior to rating urge. It is possible that greater abstinence and more smoking exposure leads to more robust BPD effects on urge, although these factors have not been directly tested.

As far as mood, there were main effects of nicotine dose such that participants who smoked nicotine cigarettes had lower ratings of depression-dejection, anger-hostility, fatigue-inertia, and total mood disturbance compared to those who smoked placebo cigarettes. Furthermore, main effects of dose expectancy were observed such that those who were told nicotine reported lower ratings of depression-dejection, anger-hostility, and total mood disturbance than those told placebo. For the tension-anxiety and vigor-activity factors there were nicotine dose by dose expectancy interactions. Similar to urge, dose expectancy had an effect on tension-anxiety in the context of placebo administration but not nicotine administration. However, dose expectancy effects were observed for vigor-activity only among those given nicotine, such that being told nicotine produced much greater vigor than being told placebo. In this case, one could conclude that smoking a nicotine cigarette under the guise that it was a placebo lessened the pharmacological effects of nicotine, resulting in an "antiplacebo" effect (see Perkins et al., 2003). Other BPD studies to date have not used the POMS or other comprehensive measures of mood, but rather have assessed one or two mood dimensions. In general, prior research has shown that denicotinized cigarettes smoked under double-blind conditions have more robust effects on craving reduction than negative affect reduction (Buchhalter et al., 2005; Rose, 2006). Our findings suggest that, in addition to nicotine, dose expectancy has important effects on mood. Given that mood states may influence motivation to smoke (Conklin & Perkins, 2005), and many smokers believe that smoking improves mood (Copeland et al., 1995), this should be further explored.

A number of main effects were observed for both the nicotine factor and dose expectancy factor on subjective ratings of the experimental cigarettes. Compared to placebo cigarettes, nicotine cigarettes led to greater rewarding effects on all 11 dimensions. Nicotine cigarettes were also rated higher on similarity to one's usual brand and dizziness. Relative to those told placebo, participants who were told nicotine reported greater rewarding effects of the cigarette on 9 of 11 dimensions. Interestingly, there were no nicotine dose by dose expectancy interactions on subjective ratings of the cigarettes, which was also the case in the Kelemen and Kaighobadi (2007) study (with the exception of cigarette strength, which was not measured in this study). The effect sizes for nicotine effects were generally larger than the effect sizes for dose expectancy effects, also similar to Kelemen and Kaighobadi (2007). Other studies have also shown effects of nicotine dose and dose expectancy on smoking satisfaction and/or liking (Perkins et al., 2008; Perkins et al., 2004). The present study adds to the data by demonstrating that the perceived rewarding effects of ciga-

rette smoking are highly influenced by both nicotine dose and dose expectancy and have additive but not interactive effects. This could have implications for the use of denicotinized cigarettes as a treatment tool, or a component of expectancy challenge interventions.

Smoking behavior was also affected by both nicotine dose and dose expectancy. Participants who smoked a nicotine cigarette spent significantly more time smoking than those who smoked a placebo cigarette. For the total number of puffs an interaction was observed. Among participants given nicotine, those who were told nicotine took more puffs than those told placebo, but among those given placebo, those told nicotine took fewer puffs than those told placebo. Although the effects of the manipulations on smoking behavior is interesting in its own right, smoking behavior could have confounded the effects of the experimental manipulations on the other dependent measures. To investigate this possibility, we reran all analyses including the number of puffs and/or smoking duration as covariates in the ANCOVA models for all dependent measures. Doing so did not have any effects on the subjective outcomes (urge, POMS, ratings of the cigarettes), the dose expectancy effect on false alarm RVIP outcomes, or the nicotine dose effects on RVIP hits. However, for RVIP reaction time and RVIP sensitivity adding these covariates reduced the significant main effects of nicotine dose to statistical trends. Studies that control smoking exposure across the conditions of the BPD would eliminate systematic differences in smoking exposure that could confound the manipulations, but this would likely result in less naturalistic smoking conditions.

The findings in this study and others suggest that the expected dose of the cigarette influences responses to smoking, but this does not shed light on the influence of smokers' underlying beliefs about the effects of smoking on smoking outcomes (i.e., response expectancies). Future studies could attempt to directly manipulate the expected effects of smoking to evaluate their causal role in smoking outcomes. Furthermore, future studies could assess baseline self-reported response expectancies to test theoretical propositions regarding the mechanisms underlying dose expectancy effects. This is especially important given data that suggests that smokers may have misperceptions about the effects of nicotine obtained via nicotine replacement products (e.g., Bansal, Cummings, Hyland & Giovino, 2004). Little is known about smokers' beliefs about the contributions of nicotine to the effects of smoking and measures that assess these beliefs need to be developed and validated.

It is important to note that 18% of participants reported smoking a type of cigarette different from the intended dose expectancy manipulation. The highest rates of disbelief were in the given placebo and told nicotine condition (i.e., 36%), followed by the given nicotine and told placebo condition (15%). These rates are different than the Juliano and Brandon (2002) study, in which the highest rates of disbelief were in the given nicotine and told placebo condition. There are important differences between the present study and this prior BPD study. Central to the issue of the credibility of the dose expectancy manipulation is that the present study used cigarettes with about half as much nicotine as those used Juliano and Brandon (2002). Furthermore, in the present study the dose expectancy manipulation was delivered by computer. Moreover, in the present study we assessed the believability of the dose expectancy manipulation with an item that had an equal number of options for nicotine and placebo. This is an improvement over the 6-point scale used in Juliano and Brandon (2002) that had only one placebo option (0 mg nicotine) and five nicotine options (.06 to 1.7 mg). Perkins et al. (2008) also found higher rates of disbelief in the in the conditions involving deception (27.5–32.5%) than the conditions not involving deception (12.5%–20%). Kelemen and Kaighobadi (2007) reported overall lower rates of disbelief and also used *Quest* cigarettes with 0.6 mg nicotine, but placed the experimental cigarettes in commercially labeled packs that were consistent with the dose expectancy manipulation, and used a delayed debriefing procedure. We also used a delayed debriefing procedure to reduce the possibility that participants would have been tipped off about the deception in the study. It is also important to note that the question about cigarette type was not asked until the end of the experiment, and thus may not reflect what participants believed at the time they smoked the cigarette. Future work in this area should continue to improve the credibility of the dose expectancy manipulation as well as its assessment.

This study did not have a nonsmoking control group and thus the absolute effects of smoking exposure independent of nicotine dose or dose expectancy cannot be evaluated. Perkins and colleagues (2008) included a no-smoking control group along with the BPD conditions and compared participants who did not smoke to those who were told and given a placebo cigarette. They concluded that the smoking ritual regardless of dose expectancy or nicotine dose attenuated negative affect relative to a no smoking condition in the context of a negative mood induction but not a positive mood induction. An evaluation of pre- to postsmoking change scores on the POMS-SF in the present study showed that for four out of five factors and total mood disturbance, mood worsened after smoking in the told and given placebo condition (vigor-activity was unchanged). Our urge data revealed substantial urge reduction in groups that were either given nicotine or told nicotine (anywhere from 2.21 to 3.46 unit reduction on a 7-point scale). However, urge in the told and given placebo condition was nearly unchanged after smoking (.19 unit decrease). Thus, it does not appear that individuals in this study who knowingly smoked a placebo cigarette experienced any absolute benefit from it; albeit it is possible that they could have been worse off if no smoking took place. Given the suggestion that denicotinized cigarettes may be a useful quitting tool (Rose, 2006), more research is needed to determine the contextual factors that determine how smokers respond when they knowingly smoke denicotinized cigarettes.

It should be noted that we relied on self-report to assess recent smoking exposure on the day of the experimental manipulation. Participants were instructed to abstain from smoking for 3 hours prior to the appointment time. Upon arrival they were asked when they last smoked, and five

participants who reported smoking in the prior 3 hours were rescheduled. We informed participants that we would assess recent smoke exposure with a CO test to encourage them to comply, but we did not use the CO results to exclude participants. Thus one potential limitation of the study is that participants could have misreported recent smoking. Future studies should better control for recent smoking exposure. Another potential limitation is that reactions to the unfamiliar cigarettes used in this study may not generalize to conditions in which participants are smoking their usual brand of cigarette.

This study provides additional evidence that nicotine plays an important role in the cognitive and rewarding subjective effects of cigarette smoking. This study also provides preliminary evidence that dose expectancy influences the cognitive performance enhancement experienced from smoking, and additional evidence that it influences smoking urge, mood and other subjective rewarding effects of cigarettes (e.g., improved concentration). Future studies should continue to evaluate the role of nonnicotine factors such as expectancy in the persistence of cigarette smoking and its effective treatment. There is preliminary evidence that smokers' expectancies for smoking (Copeland & Brandon, 2000) are modifiable and that challenging the validity of these beliefs may affect smoking motivation (Copeland & Brandon, 2000). Future research is needed to better characterize the expectancies that smokers have about nicotine, especially in the context of smoking. Furthermore, studies that attempt to directly manipulate nicotine dose and/or response expectancies could shed light on their causal role in both the effects of cigarette smoking and the effectiveness of nicotine replacement products (Fucito & Juliano, 2007; Perkins et al., 2009). Research should also continue to evaluate the effects of dose expectancies and response expectancies on other types of cognitive performance. A greater understanding of expectancy effects will guide the development of expectancy based interventions and will no doubt improve our overall understanding of clinical outcomes.

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Received September 1, 2010
Revision received December 19, 2010
Accepted December 26, 2010 ■

Original Investigation

Effects of 24 Hours of Tobacco Withdrawal and Subsequent Tobacco Smoking Among Low and High Sensation Seekers

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Received August 26, 2010; accepted April 21, 2011

Abstract

Introduction: Previous studies have indicated that high sensation seekers are more sensitive to the reinforcing effects of nicotine, initiate smoking at an earlier age, and smoke greater amounts of cigarettes. This study examined the influence of sensation-seeking status on tobacco smoking following deprivation in regular tobacco users.

Methods: Twenty healthy tobacco-smoking volunteers with low or high impulsive sensation-seeking subscale scores completed 2 consecutive test days per week for 3 consecutive weeks. Each week, a range of self-report, performance, and cardiovascular assessments were completed during *ad libitum* smoking on Day 1 and before and after the paced smoking of a tobacco cigarette containing 0.05, 0.6, or 0.9 mg of nicotine following 24 hr of tobacco deprivation on Day 2. In addition, self-administration behavior was analyzed during a 2-hr free access period after the initial tobacco administration.

Results: In high sensation seekers, tobacco smoking independent of nicotine yield ameliorated deprivation effects, whereas amelioration of deprivation effects was dependent on nicotine yield among low sensation seekers. However, this effect was limited to a small subset of measures. Subsequent cigarette self-administration increased in a nicotine-dependent manner for high sensation seekers only.

Conclusions: Compared with low sensation seekers, high sensation seekers were more sensitive to the withdrawal relieving effects of nonnicotine components of smoking following 24 hr of deprivation on selective measures and more sensitive to nicotine yield during subsequent tobacco self-administration. These results are consistent with studies suggesting that factors driving tobacco dependence may vary as a function of sensation-seeking status.

Introduction

Sensation seeking is defined as the seeking of varied, novel, complex, and intense sensations and experiences and the willingness to take physical, social, legal, and financial risks for the sake of such experience (Zuckerman, 1994). It is commonly reported that high sensation seekers initiate drug use at an earlier age, use greater amounts of drugs, are more likely to develop problems related to drug use, and are less likely to remain abstinent following drug treatment (reviewed in Zuckerman, 2007). Sensation-seeking scores among drug-naive adolescents predict sensitivity to the reinforcing effects of stimulant and sedative drugs as young adults (Kelly et al., 2009), suggesting that adolescents high in sensation seeking may be at greater vulnerability to repeated drug use. A growing body of literature also suggests that high sensation-seeking young adults are more sensitive to the reinforcing and other behavioral effects of a range of drugs including alcohol (Fillmore, Ostling, Martin, & Kelly, 2009; Magid, Maclean, & Colder, 2007), hallucinogens (Khavari, Mabry, & Humes, 1977), and stimulants (Bowling & Bardo, 1994; Kelly et al., 2006; Stoops et al., 2007). Tailoring prevention materials for high sensation seekers has been shown to increase intervention efficacy (e.g., Palmgreen, Donohew, Lorch, Hoyle, & Stephenson, 2001).

Several studies have examined the role of sensation seeking during various stages of nicotine/tobacco use. High sensation seekers are more likely to use tobacco during adolescence (Andrucci, Archer, Pancoast, & Gordon, 1989; Frankenberger, 2004). Perkins et al. (2000, 2008b) found that high scores on several personality dimensions related to sensation seeking (e.g., novelty seeking, experience seeking, disinhibition) were related to nicotine choice and increased scores on verbal reports of the reinforcing and aversive effects of an acute dose of nicotine among nonsmokers. However, individual differences in sensitivity to the behavioral effects of nicotine during acute nicotine exposure

doi: 10.1093/ntr/ntr102
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were limited to initial exposures, as this relationship was not replicated in regular smokers in this study. Among regular smokers, sensation seeking has been linked to a number of tobacco use variables, including craving response to smoking cues (Doran, Cook, McChargue, & Spring, 2009), magnitude of withdrawal effect following tobacco deprivation in ratings of negative affect and anhedonia (Carton, Le Houezec, Lagrue, & Jouvent, 2000; Leventhal et al., 2007), and higher tobacco relapse rates after a quit attempt (Kahler, Spillane, Metrik, Leventhal, & Monti, 2009), suggesting that sensation seeking plays a role in the initiation, escalation, and maintenance of tobacco use behavior.

Fewer studies have examined the role of sensation seeking as a predictor of nicotine dependence among regular smokers. Carton, Jouvent, and Widlocher (1994) found that after controlling for duration and frequency of smoking, subject-rated FTND scores correlated with experience seeking and disinhibition subscales of the Sensation-Seeking Scale in regular tobacco smokers. While consistent with a conclusion that high sensation-seeking chronic tobacco users may be more tobacco dependent than low sensation seekers, interpretation of these data must be tempered by the exclusive reliance on self-report measures, the absence of objective measures of tobacco deprivation, and a lack of experimental manipulation of the level of nicotine deprivation.

The purpose of this study was to examine the influence of sensation seeking on the effects of the nicotine yield of tobacco smoke (0.05, 0.6, and 0.9 mg) following tobacco deprivation across a range of self-administration, self-report, performance, and cardiovascular measures in a sample of regular tobacco users. We hypothesized that the deprivation period would engender nicotine deprivation effects on self-report, performance, and cardiovascular function and that tobacco cigarette smoking would ameliorate deprivation effects in a nicotine-yield dependent manner. Furthermore, based on previous reports of increased tobacco dependence among high sensation seekers, we hypothesized that high sensation seekers would show (a) greater effects of deprivation, (b) enhanced sensitivity to nicotine-yield and nonnicotine components of denicotinized cigarettes following tobacco deprivation, and (c) higher rates of cigarette smoking self-administration.

Methods

Participants

Twenty healthy adult tobacco-smoking volunteers (10 females, ages 18–38), recruited through advertisements placed on the University of Kentucky campus and in the local community, completed a six-session study that was approved by the University of Kentucky Medical Institutional Review Board. Volunteers responding to advertisements completed a brief telephone interview or an Internet-based questionnaire addressing general medical and legal status and the Impulsive Sensation-Seeking Scale of the Zuckerman–Kuhlman Personality Questionnaire (ZKPQ; Zuckerman, Kuhlman, Joireman, Teta, & Kraft, 1993). Those reporting good health and having Impulsive Sensation-Seeking Scale (IMP/SS) scores that fell in the upper (i.e., high sensation seekers: males ≥ 14, females ≥ 13) or lower (i.e., low sensation seekers: males ≤ 7, females ≤ 6) quartile of scores from a distribution of 2,969 college students (provided by M. Zuckerman, personal communication) were invited to participate in the study. The study sample size of 20 was chosen based on effect sizes from similar studies examining sensation-seeking group differences in the pharmacological effects of stimulant drugs (e.g., Kelly et al., 2006; Stoops et al., 2007).

During an orientation and medical screening day, volunteers completed a battery of medical and psychological questionnaires, including the Eysenck Personality Inventory (EPI; Eysenck & Eysenck, 1964), Beck Depression Inventory (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), locally developed Attention Deficit Hyperactivity Disorder (ADHD) and conduct disorder checklists, the Brief Symptom Inventory (Derogatis, 1993), and Form V of the Zuckerman Sensation-Seeking Scale (Zuckerman, Eysenck, & Eysenck, 1978), as well as blood chemistry, liver function, and urinalysis tests. Tobacco-smoking status was verified by assessing breath carbon monoxide (CO) levels. To be eligible to participate, subjects were required to have a CO level ≥ 12 ppm. Volunteers were excluded if they had a history of or current significant medical illness (e.g., cardiovascular disease, neurological or psychiatric disorder), excessive use of alcohol or caffeine, regular use of other drugs, pregnant or breastfeeding status, or any other condition that would increase risk for study participation. During a separate training session, participants practiced the study tasks until performance was consistent and accurate across consecutive trials. Twenty-two volunteers initiated the study; two participants dropped out for reasons unrelated to the study, and data from these two participants were not included in the final analysis.

Design

A double-blind, placebo-controlled, randomized design was used to examine the behavioral effects of nicotine yield (0.05 [low-yield nicotine cigarette was used as a "placebo" control], 0.6, and 0.9 mg) and time (pre- and postexperimental cigarette) in low and high sensation seekers following 24 hr of tobacco deprivation. A separate analysis of the effects of the 24-hr deprivation manipulation was also conducted.

Schedule

Each participant completed two consecutive test days per week for three consecutive weeks. At the start of test days, which occurred at the same time each day, participants answered open-ended questions regarding sleep, medication use, eating behavior, and health status during the preceding 24 hr and completed field sobriety, breath (Alcohol Sensor III, Intoximeters, Inc.; piCO Carbon Monoxide Monitor, Bedfont Scientific), and urine tests (cocaine, benzodiazepine, barbiturate, marijuana, amphetamine, and opiate drug use using OnTrak TesTstik, Varian, Inc.; pregnancy using Clearview HCG II, Unipath, Ltd). Thirty-minute sessions were completed on Day 1 following *ad libitum* smoking, on Day 2 following 24 hr of tobacco deprivation, as verified by breath (CO levels ≥ 10 ppm were required to complete Day 2 testing), and then repeated on Day 2 following paced smoking of eight puffs from one experimental cigarette. The paced smoking procedure was adapted from Kelly, Foltin, Rose, Fischman, and Brady (1990) and consisted of a 3-s preparation interval, a 3-s inhalation interval, and a 14-s exhale and rest period. This procedure was

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repeated eight times for each experimental cigarette. Following the postsmoking session, additional experimental cigarettes containing the nicotine yield administered that day could be self-administered for 2 hr. All cigarettes were smoked using a mouthpiece connected at the front and rear with PVC tubing to a volumetric transducer. The flow of air through the mouthpiece was measured to determine the duration and volume of each puff.

Upon completion of testing each week, subjects received $60. Upon successful completion of all three 2-day testing occasions, subjects received an additional $180 bonus plus task performance earnings. Total earnings were approximately $365; there were no group differences in earnings.

Drug

Commercially available cigarettes delivering 0.05 (Quest® Step 3), 0.6 (Quest® Step 1), and 0.9 mg (Kent®) of nicotine were prepared with black tape covering the brand name, so that all cigarettes looked the same, regardless of nicotine content. Doses (i.e., nicotine yield) were administered in a randomized order across the 3 weeks of the study.

Session Measures

During each session, self-report questionnaires (e.g., Foltin & Fischman, 1991), psychomotor and cognitive tasks (e.g., Roache, 1991), and cardiovascular measures were completed in the following order.

Wisconsin Smoking Withdrawal Scale

This 28-item questionnaire yields ratings on seven subscales: anger, anxiety, concentration, craving, hunger, sadness, and sleep on scales from 0 to 4. Each item was rated along a 5-point scale, from "Strongly disagree" to "Strongly agree." This scale was used to assess the global effects of tobacco withdrawal (Welsch et al., 1999). Participants completed the Wisconsin Smoking Withdrawal Scale (WSWS) during the *ad libitum* session and during the pre-smoking session occurring following 24-hr deprivation.

Visual Analog Scale

Participants rated items (I feel stimulated, stressed, sedated, hungry, anxious, light-headed, thirsty, sleepy, sick to my stomach, down, high, drug effect, and I like the drug effect) presented individually on the computer by marking a 100-unit line anchored on the extremes by "Not at all" and "Extremely."

Profile of Mood States

Participants completed an experimental version of the Profile of Mood States (POMS) (derived from McNair, Lorr, & Droppleman, 1971) consisting of 72 adjectives rated along a 5-point scale, from "not at all" to "extremely," which yielded scores on 10 clusters: anxiety, depression, anger, vigor, fatigue, confusion, friendliness, elation, arousal, and total positive.

Addiction Research Center Inventory

The 49-item short form of the true–false inventory yielded information on five dimensions: lysergic acid diethylamide (LSD) Scale, Amphetamine (A) Scale, Benzedrine Group (BG) Scale, Morphine –Benzedrine Group (MBG) Scale, and the Pentobarbital, Chlorpromazine, Alcohol Group (PCAG) Scale (Martin, Sloan, Sapira, & Jasinski, 1971).

Minnesota Smoking Withdrawal Scale

Participants rated items associated with tobacco withdrawal (i.e., craving, difficulty concentrating, restlessness, headaches, drowsiness, and gastrointestinal symptoms) on a scale from 0 (*not present*), to 3 (*severe intensity*; Hughes & Hatsukami, 1986). This scale was used to assess the immediate effects of tobacco withdrawal and subsequent effects of smoking a cigarette.

Cardiovascular Assessment/Math Stress Task

Oscillometric systolic and diastolic blood pressure measures and heart rate were obtained (Sentry II, NBS Medical) before, during, and after completion of math addition problems. Subjects were paid 2 cents for each correct answer. The difficulty level of the addition problems (number of digits) and the duration of the time given to answer the problems were systematically manipulated based on participant performance accuracy in order to maintain a standard level of performance, regardless of math ability or experimental manipulations. As such, performance on this task was not analyzed.

Repeated Acquisition of Response Sequences

The acquisition phase required the subject to press four keys (1, 3, 7, and 9) on a numeric keypad to learn a new 10-response order (a "chain"). When the first correct key in the sequence was pressed, a "position" counter on the screen increased from 0 to 1. The position counter then increased by one each time the subject pressed the correct key in a given position in the sequence, but did not change if the subject pressed the incorrect key. If any key other than the correct key was pressed, a brief time out (blank screen) occurred. When the subject pressed the 10th and final key in the sequence, a "points" counter increased by one and the position counter was reset to 0, indicating that the first response in the order was again required. Subjects had 180 s to complete as many chains as possible. During the performance phase, the 10-response order remained the same across sessions and subjects had 60 s to complete as many chains as possible. The primary dependent measures for this task were the number of chains completed, the number of errors committed, and the percentage of correct responses. For the acquisition version of the task, the index of curvature for the number of errors committed was also calculated to determine how efficiently the 10-response order was acquired. Stimulant drug effects on repeated acquisition of response sequences (RA) task performance have been reported to vary as a function of sensation seeking status (Stoops et al., 2007).

Digit–Symbol Substitution Task

Participants completed a 1.5-min computerized version of the Digit–Symbol Substitution Task (DSST) adapted from McLeod, Griffiths, Bigelow, and Yingling (1982). Trial completion rate and accuracy was monitored on this psychomotor task.

Rapid Information Processing Task

Participants completed a 5-min computerized version of the rapid information processing task (RIP) (Fillmore et al., 2005). At the start of this task, single digits were presented in the center of the monitor at a rate of 90 digits/min, and subjects were instructed to press a key whenever three consecutive even or odd digits were presented. Each digit was displayed on the screen for 67 ms with an ISI of 600 ms. Following correct responses, the speed of digit presentation was increased, and following incorrect responses or missed signals, the speed of digit presentation was decreased. Information-processing capacity (working

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memory) was determined based on the rate of digit presentation and signal detection accuracy (proportion of hits).

Tobacco Self-Administration Smoking Topography Measures

Smoking topography measures during the 2-hr smoking period included number of cigarettes smoked, puffs per cigarette, and total puff volume and duration. Participants also completed the Cigarette Rating Questionnaire (CQ; Westman, Levin, & Rose, 1992), which assesses tobacco smoke characteristics, including: good taste, calmed, awake, reduced hunger, irritability, nauseated, dizzy, reduced craving, and throat/chest sensations on 100-unit Visual Analog Scale (VAS). The CQ was administered twice, once after the experimental cigarette session and again after *ad libitum* smoking.

Data Analysis

Sensation-seeking group differences in demographic variables and CO levels were analyzed using independent *t*-tests. A preliminary analysis of the 24-hr deprivation period was conducted using a three-way mixed-model analysis of variance (ANOVA) with sensation-seeking status as a between-subjects factor, and test week (1–3) and session (pre- and postdeprivation) as within-subject factors. The results of the experimental cigarette administration were analyzed using a two-way mixed-model analysis of covariance (ANCOVA) with the 24-hr deprivation baseline used as the covariate, sensation-seeking status as the between-subjects factor, and nicotine yield (0.05, 0.6, 0.9 mg) as the within-subject factor. Type I error was minimized by examining significant interactions using simple-effects models and examining main effects using the Tukey–Kramer adjusted differences of least-squared means. *Ad libitum* smoking topography was analyzed using a 2 × 3 (sensation seeking × nicotine yield) mixed-model ANOVA. Due to equipment malfunction, topography measures were not available for two participants. Since collapsing across symptoms and analyzing only a total score can obscure specific effects during assessment of tobacco craving and withdrawal, analyses of individual assessment items has been recommended (Hughes & Hatsukami, 1998; Shiffman, West, & Gilbert, 2004). As such, items from the Minnesota Smoking Withdrawal Scale (MNWS), WSWS, and VAS were analyzed individually. All results were considered significant at $p \leq .05$. Analyses were conducted using SAS version 9.1.

Results

Demographics

Table 1 presents participant demographics. Tobacco use frequency and nicotine yield of preferred cigarettes were similar in both groups. No participant reported being a regular smoker of either brands of cigarettes used in the study (i.e., Quest or Kent brands). No drug use or pregnancy was detected during daily urinalysis testing. As expected, two-sample *t*-tests confirmed that ZKPQ scores were significantly greater for high sensation seekers. Groups were also significantly different in total scores on form V of the Zuckerman Sensation-Seeking Scale ($p < .01$), the impulsivity subscale of the Addiction Research Center Maturation Scale ($p < .05$), and extraversion on the EPI ($p < .01$), but did not differ on any of the other screening questionnaires examining symptoms of behavioral undercontrol (e.g., ADHD or conduct disorder), mood disorders, or psychiatric symptoms.

Deprivation Effects

The 24-hr deprivation period was used to engender a consistent level of tobacco deprivation upon which to examine the effects of smoking and nicotine yield. Compliance with the deprivation intervention was verified by breath CO (Table 1). There were no significant group differences in breath CO levels during

Table 1. Participant Demographic Information and CO Levels Before and Following 24 Hr of Tobacco Deprivation

	Low SS			High SS		
	Mean	*SD*	Range	Mean	*SD*	Range
ZKPQ IMP/SS score	5.0	1.2	3–7	15.8***	2.1	13–19
Age	25.1	7.0	18–38	21.0	2.5	18–24
Height (inches)	67.8	4.6	62–74	71.1	4.7	64–77
Weight (lbs)	165.6	41.9	110–260	190.5	43.2	115–250
Education	14.1	2.1	12–17	13.5	1.4	12–17
Tobacco (cigarettes/day)	15.5	6.9	10–30	15.0	4.1	10–20
Nicotine yield (mg/cigarette)	0.9	0.3	0.5–1.6	1.0	0.3	0.6–1.6
Caffeine (mg/day)	139.1	167.5	0–580	95.0	106.5	0–350
Alcohol (drinks/week)	8.1	6.7	0–23	6.9	7.5	0–20
Marijuana (occasions/month)	0.3	0.9	0–3	0.1	0.3	0–1
CO level						
0-hr deprivation	24.8	9.6	13–47	21.4	7.4	14–35
24-hr deprivation	3.9###	1.1	3–6	5.1###	1.9	3–9

Note. Total sample size was an *N* of 20 (10 per group). CO levels varied as a function of deprivation in both groups; however, there were no significant group differences in CO levels. CO = carbon monoxide; IMP/SS = Impuslive-Sensation Seeking subscale; SS = Sensation Seeking group; and ZKPQ = Zuckerman-Kuhlman Personality Questionnaire.

***$p<.001$ (difference between Low and HIgh SS); ###$p<.001$ (difference between 0-hr and 24-hr deprivation).

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ad libitum smoking or after 24 hr of smoking deprivation, as determined during intake assessments on test days.

Figure 1 displays the results of the MNWS (Table 2) and the WSWS, which were used to examine the effects of 24 hr of smoking deprivation. As expected, significant increases on both measures were observed following 24 hr of deprivation. In addition, deprivation-induced changes were also observed on most of the other self-report measures (Table 2). No differences on self-report measures of 24-hr deprivation effects were observed as a function of sensation-seeking status.

The effects of 24-hr deprivation on RIP and DSST task performance varied as a function of sensation-seeking status. Significant sensation seeking × session interactions were observed on RIP proportion correct, $F(1,18) = 6.904$, $p < .05$, and correct commissions, $F(1,18) = 9.669$, $p < .05$, and DSST incorrect responses, $F(1,18) = 6.891$, $p < .05$. Simple effects indicated that deprivation-induced impairment occurred only among high sensation seekers. Deprivation-induced increases were also seen on errors during the acquisition phase of the RA task ($p < .01$) and correct responses on the performance phase of the RA task, but these effects did not differ as a function of sensation-seeking status.

The effects of 24-hr deprivation on systolic blood pressure also varied as a function of sensation-seeking status. Follow-up testing indicated that systolic pressure decreased following deprivation for high sensation seekers, only ($p < .05$). Deprivation also decreased cardiovascular measures of heart rate and diastolic blood pressure, but these effects were not different among low and high sensation seekers.

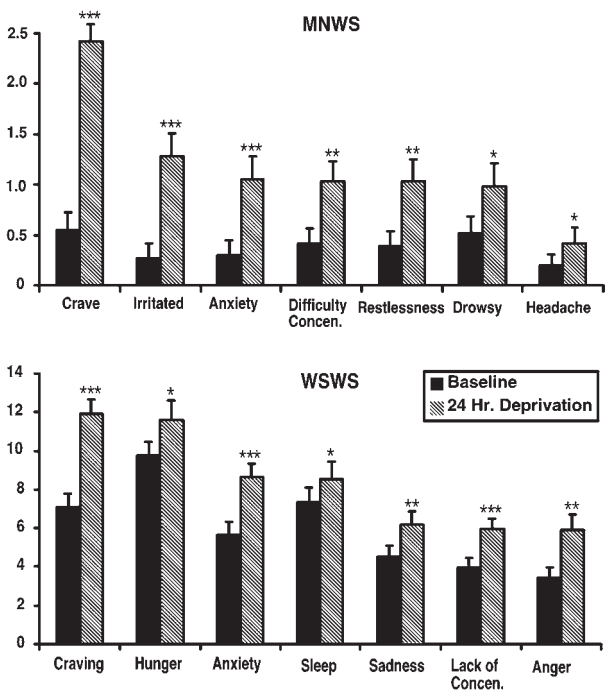


Figure 1. Presents significant effects of the 24-hr deprivation period on the Minnesota Smoking Withdrawal and Wisconsin Smoking Withdrawal Scales. Significant increases in mean subject-rated withdrawal effects were found on both measures. *$p < .05$, **$p < .01$, ***$p < .001$. Error bars represent 1 *SE*.

Smoking Effects

Subjective Measures

Minnesota Smoking Withdrawal Scale. Effects of nicotine yield on ratings of restlessness from 24-hr deprivation baseline level varied as a function of sensation-seeking status (Table 2, Figure 2, Panel A). Simple effects analyses of the interaction indicated that decreases in ratings occurred as a function of nicotine yield in low sensation seekers alone, while high sensation seekers reported lower ratings of restlessness than low sensation seekers after smoking the 0.05-mg cigarette (i.e., changes in ratings among high sensation seekers occurred following smoking, regardless of nicotine yield; while changes among low sensation seekers were dependent on nicotine yield).

Nicotine reduced ratings on other MNWS similarly among low and high sensation seekers. Ratings on the MNWS Craving scale, pooled for sensation-seeking status, are presented in Panel A of Figure 3. Ratings were significantly lower after smoking the 0.6- and 0.9-mg cigarettes when compared with ratings following the 0.05-mg cigarette. Similar effects were observed on the other MNWS.

Visual Analog Scale. Nicotine effects on VAS stimulated varied as a function of sensation-seeking status (Figure 2, Panel B). Simple effects indicated that increases in ratings occurred as a function of nicotine yield in low sensation seekers alone, while high sensation seekers reported higher ratings of stimulated than low sensation seekers after smoking the 0.05-mg cigarette ($p = .07$). These results were similar to those on MNWS restlessness, with changes in ratings among high sensation seekers dependent on smoking but independent of nicotine yield, and changes among low sensation seekers dependent on nicotine yield.

Nicotine effects on other VAS items did not differ as a function of sensation-seeking status. Panel B of Figure 3 presents the pooled effects of nicotine yield on ratings of anxious. Significant effects of nicotine yield were found, with follow-up testing indicating reductions in deprivation-induced ratings of anxious at 0.6- and 0.9-mg yields relative to 0.05 mg. Panel C of Figure 3 presents a main effect of nicotine yield on ratings of light headed. Follow-up testing indicated increases in ratings of light-headed at the 0.6- and 0.9-mg yields relative to 0.05 mg. Similar effects were found on ratings of head rush (Figure 3, Panel D), stimulated, like drug, pleasant feeling, and sedated.

Profile of Mood States. Nicotine effects on POMS elation varied as a function of sensation-seeking status (Figure 2, Panel C). Simple effects indicated nicotine-induced increases in both low and high sensation seekers, with a larger magnitude increase observed in low sensation seekers. In addition, high sensation seekers reported higher ratings of elation than low sensation seekers after smoking the 0.05-mg cigarette ($p = .07$). While group differences were found on ratings of vigor, with high sensation seekers reporting higher ratings than low sensation seekers, no main effects of nicotine yield or nicotine yield by group interactions were observed.

Nicotine effects on other POMS scales did not differ as a function of sensation-seeking status. Figure 3 (Panel E) presents the pooled ratings of total positive. A main effect of nicotine yield was observed, with ratings at the 0.9-mg nicotine yield significantly increased compared with the 0.05-mg yield. Similar effects were observed on ratings of friendliness. Nicotine yield-dependent decreases were observed on ratings of anxiety, anger, and depression.

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Table 2. Presents the *F* Ratios of the 24-Hr Deprivation Analysis of Variance (First Column) and the Separate and Interactive Effects of Nicotine Yield and Sensation Seeking During the Experimental Cigarette ANCOVA (Columns 2–4)

		Experimental cigarette analysis		
Measure	24-hr deprivation, *df* (1,18)	Nicotine yield, *df* (2,36)	SS, *df* (1,18)	Nicotine yield × SS, *df* (2,36)
MNWS				
Crave	82.65***	11.86***	1.54	0.54
Irritated	24.83***	1.80	1.38	0.14
Anxious	18.13***	0.89	0.05	0.07
Difficulty concentrating	20.78***	2.50	0.72	0.23
Restlessness	14.43**	2.80	0.51	3.21*
Headache	7.57*	2.93	0.09	0.15
Drowsy	7.96*	0.42	1.10	1.86
VAS				
Stimulated	9.50**	13.16***	0.38	3.76*
Head rush	5.04*	15.79***	0.03	0.31
Relaxed	21.81***	2.71	1.88	0.17
Pleasant feeling	16.01**	5.85**	2.80	0.08
Stressed	7.36*	2.78	1.12	0.88
Anxious	10.46**	6.36**	3.08	2.74
Jittery	6.28*	0.08	0.12	1.28
Sedated	1.78	6.17**	0.03	0.02
Light headed	0.01	14.26***	0.02	0.83
High	4.05	2.68	0.03	1.27
Drug effect	3.82	2.80	0.96	0.52
Like drug	4.11	3.42*	1.34	0.76
Thirsty	0.93	0.89	1.79	0.67
Sick to stomach	0.00	2.20	1.24	0.27
POMS				
Elation	26.20***	8.26***	0.44	3.39*
Total positive	18.55***	8.91***	0.88	1.93
Vigor	12.77**	2.35	6.13*	1.10
Friendliness	20.22***	3.60*	1.52	0.01
Anxiety	13.53**	5.90***	0.85	0.29
Anger	7.62*	4.99*	0.76	0.37
Confusion	8.86**	0.69	0.17	0.11
Depression	2.62	4.35*	0.56	0.00
ARCI				
MBG	25.27***	2.46	0.86	1.33
A	10.93**	0.81	3.17	0.38
BG	10.96**	1.35	5.76*	1.11
LSD	13.98**	4.62*	0.53	1.72
PCAG	18.24***	0.91	5.10*	3.32*
RA				
Acquisition				
Correct responses	1.25	1.73	0.00	0.61
Incorrect responses	9.36**	0.98	0.05	1.86
Errors index of curvature	4.21	0.43	0.27	0.24
Performance				
Correct responses	26.45***	3.91*	0.96	0.70
Incorrect responses	1.23	3.30*	0.19	3.64*
DSST				
Correct responses	0.63	2.88	2.15	1.29
Incorrect responses	1.13	0.13	7.36*	0.57
RIP				
Characters per minute	0.60	1.21	0.13	0.28
Errors of commission	0.15	1.29	1.96	0.49
Errors of omission	0.11	2.34	0.51	0.35

Table 2. Continued

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Table 2. Continued

Measure	24-hr deprivation, *df* (1,18)	Experimental cigarette analysis: Nicotine yield, *df* (2,36)	SS, *df* (1,18)	Nicotine yield × SS, *df* (2,36)
Cardiovascular				
Baseline HR	38.44***	43.30***	0.15	1.66
Baseline systolic BP	20.18***	1.35	0.27	1.34
Baseline diastolic BP	18.55***	0.74	2.61	0.56

Note. A = Amphetamine; ANCOVA = analysis of covariance; ARCI = Addiction Research Center Inventory; BG = Benzedrine Group; BP = blood pressure; DSST = Digit–Symbol Substitution Task; HR = heart rate; LSD=lysergic acid diethylamide; MBG = Morphine–Benzedrine Group; MNWS = Minnesota Smoking Withdrawal Scale; PCAG = Pentobarbital, Chlorpromazine, Alcohol Group; POMS = Profile of Mood States; RA = repeated acquisition of response sequences; RIP = rapid information processing; SS = Sensation Seeking group; and VAS = Visual Analog Scale.

*$p < .05$; **$p < .01$; ***$p < .001$.

Addiction Research Center Inventory. The effects of nicotine yield on the PCAG Scale varied as a function of sensation-seeking status, with simple effects indicating a nicotine-yield dependent decrease among high sensation seekers only. In addition, high sensation seekers reported lower PCAG scores for both the 0.05- and 0.9-mg nicotine yields compared with low sensation seekers. Group differences were also found on BG scale ratings, with high sensation seekers reporting higher ratings than low sensation seekers, but no main effects of nicotine yield or nicotine yield by group interactions were observed.

A main effect of nicotine yield was found on the LSD scale, with follow-up testing indicating that LSD scores were lower at the 0.6-mg nicotine yield compared with the 0.05-mg yield.

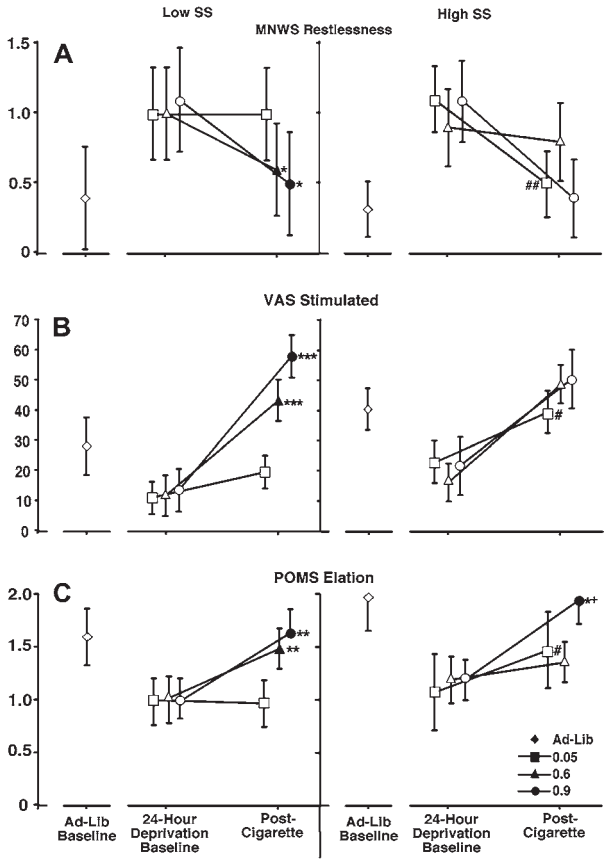


Figure 2. Presents subject-rated effects of Minnesota Smoking Withdrawal Scale Restlessness (Panel A), Visual Analog Scale Stimulated (Panel B), and Profile of Mood States Elation (Panel C) during *ad libitum* smoking baseline and following 24 hr of deprivation prior to and after cigarette administration in low and high sensation seekers. A significant nicotine-yield × sensation-seeking interaction was present, with group differences present at the 0.05-mg postcigarette assessment and greater nicotine-induced changes in low sensation seekers for all three measures. Filled circles represent a significant change from the 0.05-mg nicotine yield postcigarette measurement, while asterisks (*) represent the magnitude of that change. *$p < .05$, **$p < .01$, ***$p < .001$. Crosses (+) represent a significant difference between 0.6- and 0.9-mg nicotine yield postcigarette measurement. +$p < .05$. Pound signs (#) represent a significant SS group difference at the 0.05-mg nicotine yield. #$p < .10$, ##$p < .05$. Error bars represent ±1 *SE*. SS = Sensation Seeking group.

Cigarette rating questionnaire. Cigarettes ratings varied as a function of nicotine yield for all subscales, as evidenced by a main effect of nicotine yield. However, CQ ratings were unrelated to sensation-seeking status.

Performance Measures

RA task—acquisition. No significant effects of group or nicotine yield were observed.

RA task—performance. A significant nicotine yield × sensation seeking group interaction was observed for incorrect responses. Simple effects indicated a nicotine-yield dependent decrease in incorrect responding at both active nicotine yields in low sensation seekers, whereas incorrect responses were decreased only at the 0.6-mg nicotine yield among high sensation seekers.

A main effect of nicotine yield was present for correct responses, with increases observed at both active nicotine yields relative to the 0.05-mg yield.

Digit–Symbol Substitution Task. High sensation seekers committed more errors on the DSST compared with low sensation seekers. Nicotine yield had no effects on DSST performance.

Rapid information processing. No significant effects of group or nicotine yield were observed.

Cardiovascular Measures

Figure 3 (Panel F) presents the effects of nicotine yield on heart rate. Heart rate was increased after smoking the 0.6- and 0.9-mg cigarettes compared with the 0.05-mg yield. No differences in other cardiovascular measures were observed.

Tobacco Self-administration

No changes in total cigarettes consumed, total number of puffs, or puff duration were observed, but puff volume increased in a nicotine-dependent manner, $F(2,36.8) = 4.34$, $p < .05$]. Figure 4

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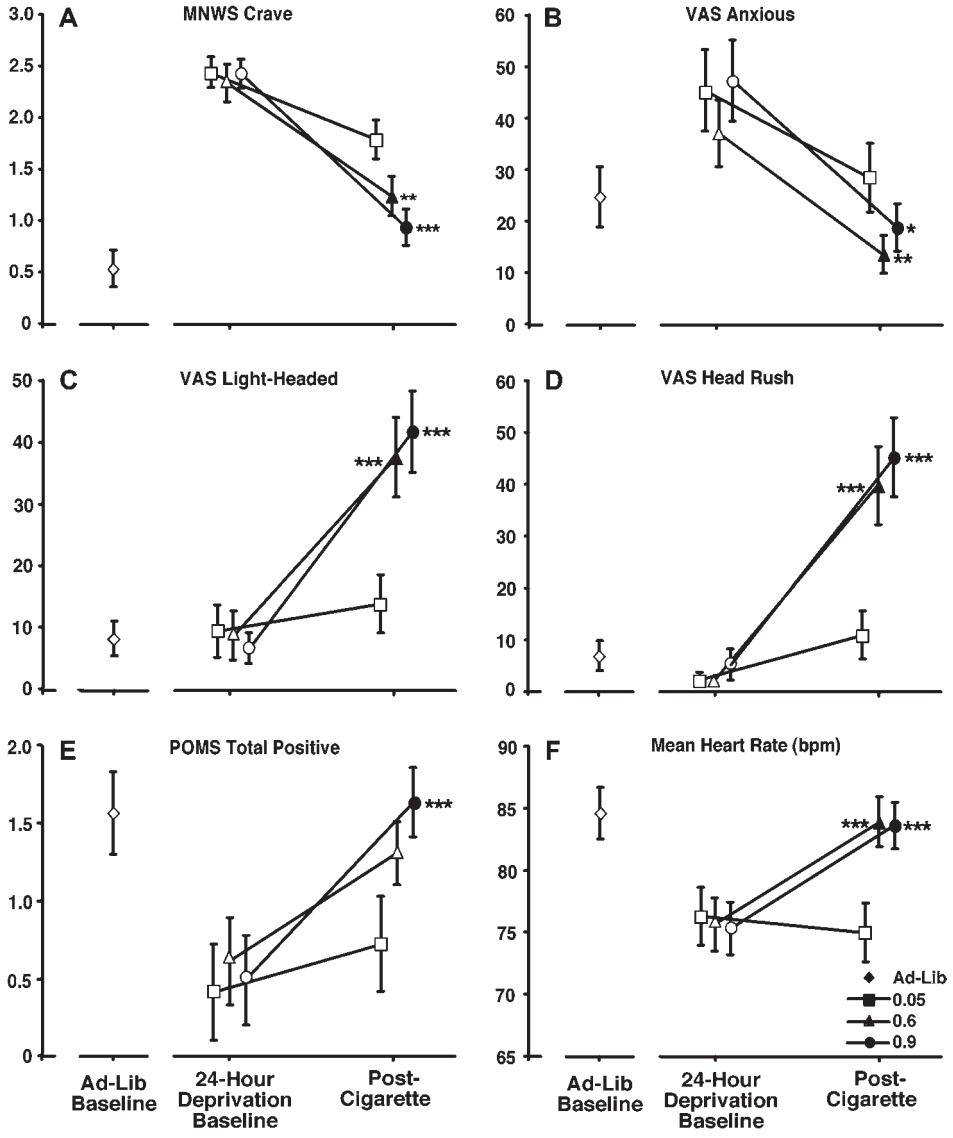


Figure 3. Presents subject-rated (Panels A–E) and cardiovascular (Panel F) measures that varied as a function of nicotine yield. Filled circles represent a significant change from the 0.05-mg nicotine yield postcigarette measurement, while asterisks (*) represent the magnitude of that change. *$p < .05$, **$p < .01$, ***$p < .001$. Error bars represent ±1 *SE*.

presents the number of cigarettes consumed and total puff volume over the 2-hr self-administration session as a function of sensation-seeking status. To examine change in tobacco self-administration as a function of active nicotine yield, a supplemental 2 × 2 ANCOVA was performed using cigarette smoking topography at the 0.05-mg yield condition as a covariate. The supplemental analysis revealed significant sensation seeking × nicotine yield interactions on number of cigarettes smoked, $F(1,20) = 5.16$, $p < .05$, total puff volume, $F(1,18) = 4.78$, $p < .05$, and puff duration, $F(1,18) = 5.47$, $p < .05$. Simple effects indicated that the number of cigarettes smoked, total puff volume, and puff duration increased during the 0.9-mg cigarette self-administration period in high sensation seekers only.

Discussion

This study examined the self-report, physiological, psychomotor, and reinforcing effects of nicotine in smoked tobacco following 24 hr of tobacco deprivation in high and low sensation seekers with similar baseline rates of cigarette smoking. Tobacco deprivation engendered expected withdrawal effects on self-report, performance, and cardiovascular measures while tobacco smoking ameliorated many of these effects in a nicotine dose-dependent manner in both low and high sensation seekers. In high sensation seekers, deprivation effects on selective measures (e.g., increases in MNWS restlessness and decreases in VAS stimulated, Addiction Research Center Inventory [ARCI], PCAG, and POMS elation) were ameliorated by tobacco smoking independent of nicotine yield, whereas amelioration of these deprivation effects were dependent on nicotine yield among low sensation seekers. This study also examined tobacco self-administration as a function of nicotine yield following 24 hr of tobacco deprivation in high and low sensation seekers. Smoking behavior increased in a nicotine-dependent manner only among high sensation seekers. Although interactions of sensation seeking and nicotine yield were limited in quantity, the significant

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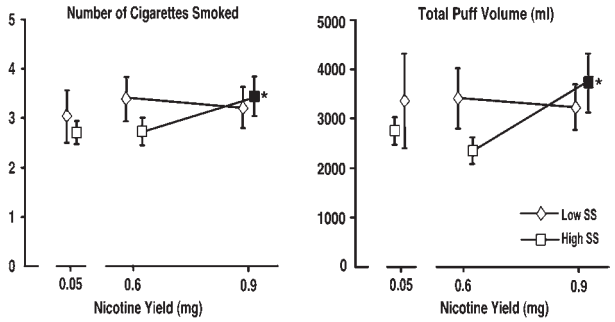


Figure 4. Presents cigarette consumption and puff topography as a function of sensation-seeking status. Filled symbols indicate significant difference between 0.6- and 0.9-mg nicotine yields, while asterisks (*) represent the magnitude of that change. *$p < .05$. Error bars represent ±1 *SE*. SS = Sensation Seeking group.

results were consistent across measures and suggest that, among smokers with similar baseline smoking rates, high sensation seekers are less sensitive than low sensation seekers to effects of cigarette nicotine yield following 24 hr of deprivation. In addition, high sensation seekers are more sensitive to nicotine yield during subsequent tobacco self-administration. Therefore, smokers high in sensation seeking may obtain more withdrawal relief from the nonnicotine effects of smoking, but may obtain more reinforcement from the nicotine effects of smoking.

Heishman, Taylor, and Henningfield (1994) have articulated the importance of establishing quantitative baseline measures of tobacco deprivation effects prior to evaluating deprivation interventions. In this study, the quantitative effects of the 24-hr deprivation manipulation were determined by comparing measures obtained during an *ad libitum* smoking with those following deprivation. Consistent with previous studies, 24 hr of tobacco deprivation increased self-report measures associated with tobacco withdrawal, impaired psychomotor task performance, and decreased heart rate and blood pressure (Evans & Drobes, 2008; Heishman et al., 1994; Hughes, 2007). These results confirm that the 24-hr deprivation manipulation in this study generated expected tobacco withdrawal effects.

Nicotine ameliorated the effects of 24 hr of deprivation in a dose-dependent manner on several verbal-report measures, heart rate, and psychomotor performance on the repeated acquisition task (see representative measures in Figure 3). In addition, nicotine increased verbal-report measures associated with the positive reinforcing effects of nicotine that were only minimally affected by tobacco deprivation (Figure 3, Panels D and E). These effects are comparable to those obtained in other tobacco deprivation studies that have examined the effects of nicotine administered via smoking and intravenous and intranasal routes (e.g, Atzori, Lemmonds, Kotler, Durcan, & Boyle, 2008; Jones, Garrett, & Griffiths, 1999; Kalman & Smith, 2005; Myers, Taylor, Moolchan, & Heishman, 2008; Parrott & Garnham, 1998).

Among smokers in the current study, the effects of tobacco deprivation varied as a function of sensation-seeking status on several measures. High sensation seekers exhibited greater decrements in RIP and DSST task performance and h*ad l*ower systolic blood pressure after 24 hr of nicotine deprivation. Previous studies have demonstrated that high sensation seekers have greater negative affect and anhedonia during tobacco abstinence (Carton et al., 2000), and higher relapse rates after initiating smoking cessation (Kahler et al., 2009). Similarly, Carton et al. (1994) found that after controlling for duration and frequency of smoking, subject-rated FTND scores correlated with experience seeking and disinhibition scores on the Sensation-Seeking Scale (Form V) in regular tobacco smokers. The deprivation-related findings did not match those of Carton et al. with regards to high sensation seekers displaying greater negative affect and anhedonia. This discordance might be due to a variety of factors (e.g., level of nicotine dependence), but it is important to note that the present study was not specifically designed to evaluate the effects of tobacco deprivation per se, as no control conditions were included. However, these results do suggest that sensitivity to some tobacco deprivation effects, after controlling for tobacco smoking rates, is related to sensation-seeking status, though further research is warranted.

High sensation seekers were also less sensitive than low sensation seekers to the effects of nicotine yield in cigarettes on verbal report measures during tobacco deprivation. Another way to explain this difference is that high sensation seekers were more responsive to effects of smoking per se, independent of nicotine intake. Smoking low nicotine-yield (0.05 mg) cigarettes reduced 24-hr deprivation effects on MNWS restlessness, VAS stimulated, POMS elation (Figure 2), and ARCI PCAG in high but not low sensation seekers. Previous studies have demonstrated that cigarette smoking, independent of nicotine content, can ameliorate craving, negative mood, withdrawal, and psychomotor task performance impairment associated with tobacco deprivation (Barrett, 2010; Perkins et al., 2008a, 2010; Robinson, Houtsmuller, Moolchan, & Pickworth, 2000). The present study suggests that the conditioned negative reinforcing effects of nonnicotine components of smoking can vary as a function of sensation-seeking status.

A growing body of literature suggests that high sensation-seeking young adults are more sensitive to the reinforcing and other behavioral effects of a range of drugs including alcohol (Fillmore et al., 2009; Magid et al., 2007), hallucinogens (Khavari et al., 1977), and other stimulants (Bowling and Bardo, 1994; Kelly et al., 2006; Stoops et al., 2007). Specific to nicotine, Perkins et al. (2000, 2008b) have demonstrated that verbal reports of mood and nicotine choice behavior are correlated with several subscales of sensation seeking among healthy nonsmokers. In the current study, tobacco cigarette self-administration following 24 hr of tobacco deprivation was robust in both low and high sensation seekers. However, an increase in tobacco self-administration was observed as a function of nicotine yield among high sensation seekers only. Cigarette brands varied with nicotine yield (Quest 3 and Quest 1 cigarettes for the 0.05- and 0.6-mg nicotine yields and Kent for the 0.9-mg nicotine yield), but Cigarette Questionnaire ratings did not vary as a function of sensation-seeking status, suggesting that group differences in self-administration were related to the nicotine yields of the cigarettes. Moreover, because the nicotine yield of preferred cigarettes was not different between low and high sensation seekers, group differences in cigarette self-administration likely were not due to these study cigarettes being more similar to the preferred brands of either group. These results suggest that, rather than being more sensitive to all effects of nicotine in cigarettes, high sensation seeking smokers may be not only more sensitive to the reinforcing effect of nicotine in tobacco smoke but also

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more sensitive to the withdrawal relieving effects of the non nicotine components of cigarette smoking.

There were limitations to the present study that should be noted. First, while an extreme groups design was used to maximize the opportunity to detect interactions between sensation-seeking status and the nicotine yield of cigarettes tested, group differences in nicotine effects were observed on only a limited number of measures, and the statistical power to detect such effects was limited due to the sample size. The sample size for the present study was chosen based upon effect sizes observed in studies that examined the reinforcing and behavioral effects of amphetamine in low and high sensation seekers (e.g., Kelly et al., 2006; Stoops et al., 2007). However, it is possible that the effects of sensation-seeking status might have been more modest in this study due to the tobacco dependent status of the study population. Using effect sizes from the current study, a power analysis revealed that an increase in sample size to an *N* of 30 would increase the power to detect several key effects of interest that were not found in this study (e.g., MNWS craving, difficulty concentrating, VAS like drug, head rush, and DSST performance). Thus, future studies might need to recruit a larger sample size in order to replicate and extend the current findings. Second, group differences in extraversion and impulsivity were obtained in this study. Given established correlations between sensation-seeking status and impulsivity (Zuckerman et al., 1993), these differences are not surprising. Furthermore, supplementary analyses controlling for extraversion by including scores on that dimension as a covariate did not change any significant effects reported in this manuscript. Nonetheless, it is possible that other variables not measured in this study could have differed among groups. Third, a separate brand of cigarette (Kent) was used for the 0.9-mg nicotine yield compared with Quest 3 and Quest 1 cigarettes for the 0.05- and 0.6-mg nicotine-yields. Although a double-blind procedure was used, it is possible that cues or tobacco ingredients specific to each brand (aside from nicotine yield) may have influenced behavior. Cigarette Questionnaire ratings did not vary as a function of brand, per se, but no direct assessment was utilized to verify that participants remained blind to brand. Finally, although several nicotine yields were assessed in this study, the range tested does not encompass the full range of yields in cigarettes currently available.

Sensation seeking plays a role at multiple stages of tobacco use, including initiation, escalation, maintenance, and cessation. Previous studies investigating the relationship between sensation seeking and tobacco/nicotine have demonstrated that high sensation seekers are more likely to use tobacco during adolescence (Andrucci et al., 1989; Frankenberger, 2004), are more sensitive to the initial reinforcing and subjective effects of nicotine (Perkins et al., 2000; 2008b), display a stronger craving response to smoking cues (Doran et al., 2009), have greater ratings of negative affect and anhedonia during tobacco abstinence (Carton et al., 2000), have higher scores on ratings of tobacco dependence (Carton et al., 1994), and are more likely to resume smoking after a quit attempt (Kahler et al., 2009). Furthermore, those high in novelty seeking are more sensitive to the effects of tobacco withdrawal (Leventhal et al., 2007). The finding that high sensation seeking smokers were more sensitive to some of the withdrawal relieving effects of denicotinized cigarettes suggests that denicotinized cigarettes may be a more effective treatment in high versus low sensation seekers (e.g., Rose, Behm, Westman, & Kukovich, 2006). Tailoring prevention materials for high sensation seekers has been shown to enhance the efficacy of marijuana interventions (e.g., Palmgreen et al., 2001). Similar approaches could be used to enhance tobacco prevention and treatment interventions.

Funding

The National Institute on Drug Abuse (P50 DA05312).

Declaration of Interests

Dr Perkins reports that he has consulted with Cypress Bioscience on treatments for smoking cessation.

Acknowledgments

We would like to thank Richard Charnigo for consultation on statistical issues and Cleeve Emurian, Beth Eaves, and Ashley Yingling for their assistance with study support and data management. This work was written with full investigator access to all relevant data and was not under editorial review by a sponsor prior to submission.

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Human Mu Opioid Receptor (*OPRM1* A118G) polymorphism is associated with brain mu-opioid receptor binding potential in smokers

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Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved April 15, 2011 (received for review December 13, 2010)

Evidence points to the endogenous opioid system, and the mu-opioid receptor (MOR) in particular, in mediating the rewarding effects of drugs of abuse, including nicotine. A single nucleotide polymorphism (SNP) in the human MOR gene (*OPRM1* A118G) has been shown to alter receptor protein level in preclinical models and smoking behavior in humans. To clarify the underlying mechanisms for these associations, we conducted an in vivo investigation of the effects of *OPRM1* A118G genotype on MOR binding potential (BP_{ND} or receptor availability). Twenty-two smokers prescreened for genotype (12 A/A, 10 */G) completed two [^{11}C]carfentanil positron emission tomography (PET) imaging sessions following overnight abstinence and exposure to a nicotine-containing cigarette and a denicotinized cigarette. Independent of session, smokers homozygous for the wild-type *OPRM1* A allele exhibited significantly higher levels of MOR BP_{ND} than smokers carrying the G allele in bilateral amygdala, left thalamus, and left anterior cingulate cortex. Among G allele carriers, the extent of subjective reward difference (denicotinized versus nicotine cigarette) was associated significantly with MOR BP_{ND} difference in right amygdala, caudate, anterior cingulate cortex, and thalamus. Future translational investigations can elucidate the role of MORs in nicotine addiction, which may lead to development of novel therapeutics.

genetics | neuroimaging | tobacco

With 1 billion tobacco users worldwide, nicotine dependence has a major impact on global health. Advances in medication development for nicotine dependence require an improved understanding of the neurobiology of this complex, relapsing brain disorder (1). Although multiple neurobiological mechanisms have been implicated, a growing body of evidence points to the endogenous opioid system, and the mu opioid receptor (MOR) in particular, in mediating the reinforcing effects of drugs of abuse, including nicotine (2 5). Nicotine up regulates MOR mRNA and protein expression in brain regions important in drug reward in rodents (4, 6) and stimulates endogenous opioid release (7, 8), resulting in MOR activation and dopamine release (9).

Genetic variation in MORs can modulate the endogenous opioid system, thereby altering behavior. A common single nucleotide polymorphism (SNP) in the mu opioid receptor gene (*OPRM1* A118G) results in an amino acid exchange at a putative glycosylation site in the extracellular terminus of the MOR (10). This *OPRM1* SNP has been associated with a variety of drug dependence phenotypes in rodent and human studies (11), including nicotine reward, nicotine withdrawal severity, and smoking relapse (12 14).

Despite substantial attention to the *OPRM1* A118G in drug addiction research, the precise function of this SNP has yet to be clarified. Although the minor (G) allele was originally thought to be a "gain of function" variant, on the basis of increased affinity of MOR agonists (15), other data suggest that the G allele is associated with reduced mRNA and protein expression (16, 17). Further, knock in mice homozygous for the equivalent (112G) allele exhibit reduced MOR mRNA and protein levels in multiple brain regions, providing additional support for a "loss of function" phenotype (18).

To translate these preclinical data to humans, we conducted an in vivo investigation of the effects of *OPRM1* A118G genotype on MOR binding potential (BP_{ND} or receptor availability). Twenty two smokers prescreened for *OPRM1* A118G genotype (12 A/A, 10 */G) completed [^{11}C]carfentanil positron emission tomography (PET) imaging during two separate sessions, both occurring after overnight (14 h) abstinence (*i*) after smoking a nicotine containing cigarette and (*ii*) after smoking a denicotinized (placebo) cigarette. Twenty nonsmoking controls (10 A/A, 10*/G) balanced for age and sex completed a single PET session. On the basis of the finding of reduced protein levels of MOR in knock in mice that carry the G allele (18), we predicted that *OPRM1* G allele carriers would have reduced MOR BP_{ND} compared with those homozygous for the A allele. Furthermore, we hypothesized that the extent of displacement of the radioligand (i.e., changes in MOR BP_{ND} across the two sessions) would be reduced in smokers with the G allele (14). Regions with high MOR density (19 21), which are important in the mesolimbic reward circuits in addiction (22), were selected as regions of interest (ROIs): anterior cingulate cortex (ACC), amygdala (AMY), caudate (CAU), ventral striatum/nucleus accumbens (VST), and thalamus (THA).

Results

The BP_{ND} in the regions of interest was calculated using the multilinear graphical analysis method evaluated by the second version of the multilinear reference tissue model (MRTM2) (23). The occipital cortex (OCC) was identified as a region of interest for use as the reference region in MRTM2 modeling (24). All regression models for MOR BP_{ND} included predictors of interest (e.g., genotype, session) and controlled for age, sex, and smoking rate (for smokers only).

Author contributions: R.R., A.N., J.A.B., J.-K.Z., and C.L. designed research; R.R., A.N., and C.D. performed research; K.R., E.P.W., J.W.L., and J.L. analyzed data; and R.R., K.R., E.P.W., J.W.L., J.A.B., and C.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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Smokers Carrying the *OPRM1* G Allele Exhibit Reduced MOR BP_{ND} Compared with Those Homozygous for the A (Wild-Type) Allele. Smokers in the */G genotype group exhibited reduced MOR BP_{ND} in several of the ROIs examined. In the cross session models, MOR BP_{ND} was significantly lower in G allele carriers in bilateral AMY [left: $\beta = -0.32$ (-0.54 to -0.10), $P = 0.004$; right: $\beta = -0.25$ (-0.46 to -0.05), $P = 0.017$], left ACC [$\beta = -0.27$ (-0.48 to -0.06), $P = 0.01$], and left THA [$\beta = -0.23$

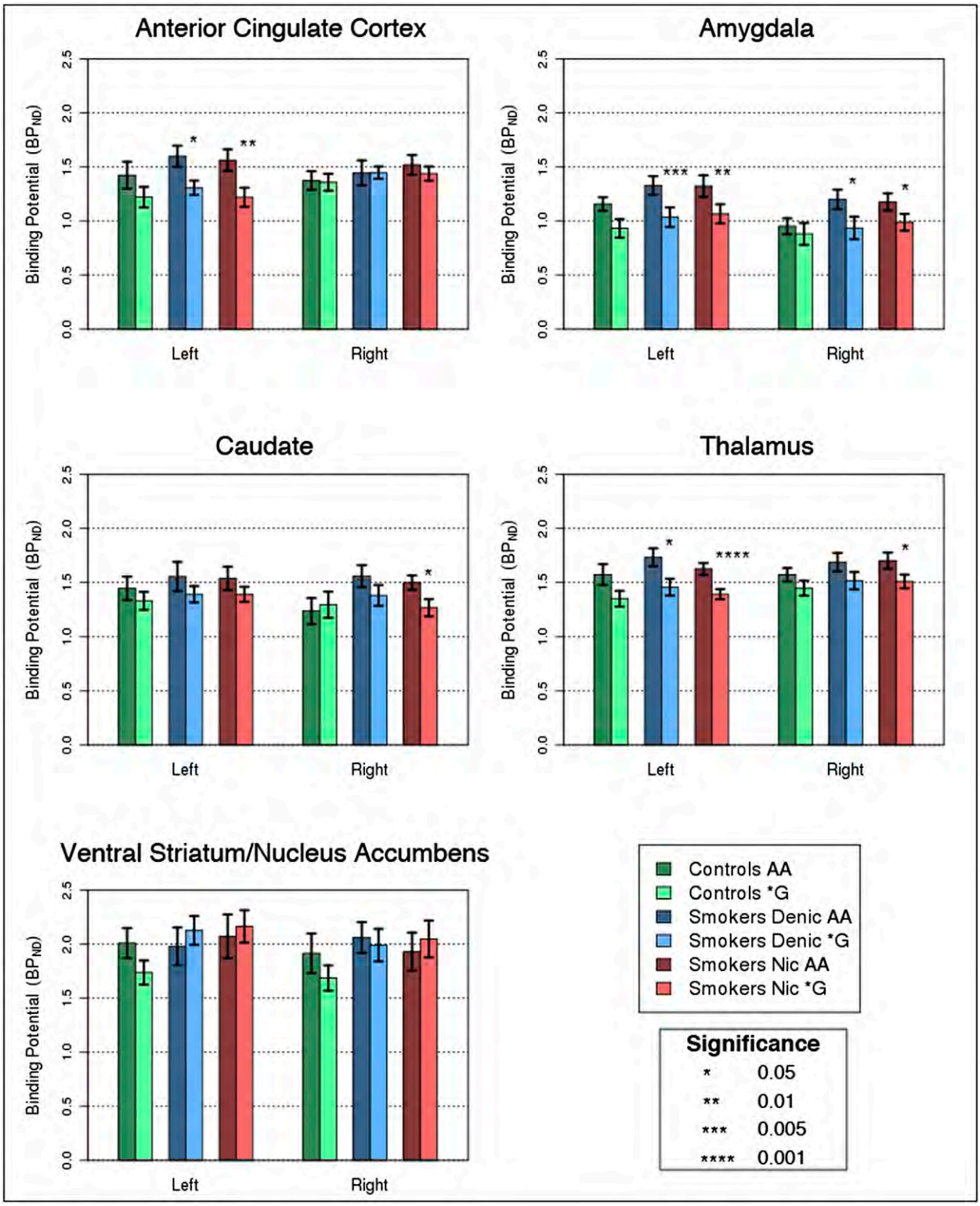


Fig. 1. Quantification of MOR BP_{ND} across the five ROIs and each hemisphere, comparing the A/A versus */G *OPRM1* groups among nonsmokers, smokers during the denicotinized cigarette session, and smokers during the nicotine cigarette session. ACC, anterior cingulate cortex; AMY, amygdala; CAU, caudate; THA, thalamus; and VST, ventral striatum/nucleus accumbens.

(−0.37 to −0.10), $P = 0.001$]. Right THA did not survive correction ($P = 0.04$). Cross session models for CAU and VST were not significant.

As shown in Fig. 1, in the nicotine cigarette session, G allele carriers exhibited reduced MOR BP_{ND} in the left AMY [β = −0.31 (−0.55 to −0.07), $P = 0.01$], left ACC [β = −0.30 (−0.53 to −0.06), $P = 0.01$], and left THA [β = −0.23 (−0.36 to −0.10), $P = 0.0004$]. Right AMY ($P = 0.03$), right CAU ($P = 0.02$), and right THA ($P = 0.02$) did not survive correction. In the denicotinized cigarette session, significant associations were identified in the left AMY (β = −0.34 (−0.57 to −0.10), $P = 0.005$) and left ACC (β = −0.24 (−0.44 to −0.04), $P = 0.018$). Right AMY ($P = 0.03$) and left THA ($P = 0.03$) did not survive correction. VST models were not significant in either session. The observed genotype associations did not differ by session (P values for genotype × session effects > 0.50). In the nonsmoking controls, genotype associations were not significant (P values > 0.10).

Changes in MOR BP_{ND} Correlate with Changes in Smoking Reward in G Allele Carriers. With respect to subjective ratings, the nicotine cigarette was rated as significantly more rewarding than the denicotinized cigarette [3.63 (SD = 1.00) versus 2.34 (SD = 0.70), respectively; $T = -4.55$, $P = 0.0002$]; subjective reward did not differ by genotype. As shown in Fig. 2, among G allele carriers, the magnitude of change in MOR BP_{ND} from the denicotinized cigarette to the nicotine session was significantly correlated (Spearman's correlation) with change in the self reported rewarding effects of these cigarettes in the right AMY ($r = 0.88$, $P = 0.001$), right CAU ($r = 0.78$, $P = 0.008$), right ACC ($r = 0.68$, $P = 0.03$), and right THA ($r = 0.65$, $P = 0.04$). There were correlations that approached significance in the left CAU ($r = 0.58$, $P = 0.08$), left THA ($r = 0.58$, $P = 0.08$), and left ACC ($r = 0.56$, $P = 0.09$). Significant correlations were not observed in the AA group (all P values > 0.10).

Comparisons of BP_{ND} in smokers (across sessions) versus nonsmokers revealed a difference only in bilateral AMY [β = −0.17 (−0.33 to −0.02), $P = 0.03$], but this effect did not survive correction. Session specific comparisons of BP_{ND} in smokers versus controls were not significant (all P values > 0.10).

Discussion

This translational study provides unique evidence linking human genetic variation in the MOR to in vivo receptor binding availability. Consistent with preclinical data (18), smokers homozygous for the wild type *OPRM1* A allele exhibited higher levels of MOR BP_{ND} in the bilateral amygdala, left thalamus, and left anterior cingulate cortex compared with those with the G allele. Among G allele carriers, the extent of subjective reward difference (denicotinized versus nicotine cigarette) was associated significantly with MOR BP_{ND} difference in right amygdala, caudate, anterior cingulate cortex, and thalamus. These regions have high MOR density (19 21) and are important in the mesolimbic reward circuits in addiction (22). Thus, the current findings may partly explain the reduced nicotine reward, withdrawal, and relapse risk associated with the A118G polymorphism (12 14, 25).

The observation of a relative reduction in MOR BP_{ND} in multiple regions among *OPRM1* G allele carriers is consistent with observations from a knock in mouse model, in which mice possess the A112G polymorphism that corresponds to the same amino acid change in humans (18). At the molecular level, the 112G mice exhibit reduced mRNA expression and MOR protein, as well as decreased [^{3}H]DAMGO binding in thalamus (18). Data from the mouse and the current human study are also consistent with evidence for *OPRM1* G allele specific decreases in MOR mRNA and protein observed in postmortem human brain (17). The present study provides in vivo evidence for this association in human smokers.

Whereas the genotype associations in this study are consistent with preclinical data, the specific mechanisms underlying the association of the *OPRM1* G allele with reduced MOR BP_{ND} are not yet clear. The possibility of a constitutive effect of the *OPRM1* genotype received limited support in our study, on the basis of the lack of significant genotype associations with MOR BP_{ND} in the nonsmokers; however, the knock in mouse data identify genotype differences in nicotine naïve animals (18). An alternate hypothesis is that long term chronic exposure to nicotine produces greater up regulation in the A/A group or down regulation in the */G group, a hypothesis that could be tested in the A112 knock in mice. It is also plausible that the MOR BP_{ND} differences are secondary to variation in endogenous opioid tone

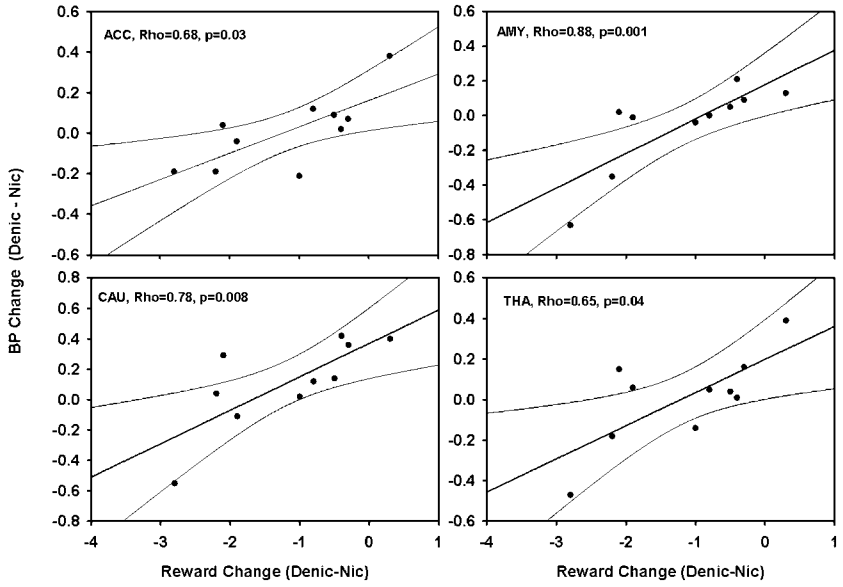


Fig. 2. Spearman's correlations of magnitude of change in MOR BP_{ND} from the denicotinized cigarette to the nicotine cigarette session with change in the self reported rewarding effects of these cigarettes.

in the two genotype groups. A postmortem brain investigation of heroin addicts linked the G allele with disruptions in the endogenous opioid neuropeptide system (26). Also supporting this mechanism, G allele carriers exhibit elevated cortisol responses to MOR blockade (27).

Our finding that subjective nicotine reward in G allele carriers is associated with MOR availability in the amygdala, thalamus, anterior cingulate cortex, and caudate supports the endogenous opioid stimulation mechanism. MORs located in the thalamus (28) and amygdala (9) decrease excitatory glutamate inputs to the striatum, which influence striatal dopaminergic firing and subsequently nicotine reward. The caudate also has a high density of MORs, which are colocalized on the inhibitory GABAergic medium spiny neurons that project from caudate putamen to the striatum (29). The projection from the caudate putamen also contributes to reward related learning (30).

Whereas the explanation for observing MOR BP_{ND} associations with reward in G allele carriers but not in the A/A group is not entirely clear, it is plausible that this observation reflects genotype differences in sensitivity associated with molecular adaptations resulting from lower MOR availability in the */G group. Indeed, evidence cited above (26, 27) suggests that compensatory alterations in endogenous opioid tone are present in this group. Altered levels of G protein coupled signaling molecules have also been observed in Neuro 2A cells transfected with the 118G *hOPRM1* (31). Additional clinical evidence suggests that carriers of the *OPRM1* 118G polymorphism exhibit increased sensitivity to pain (32), as well as increased sensitivity to psychosocial stress at both the subjective and neural levels (33). Interestingly, during the stress challenge, increased neural activity in the G allele carriers was observed in the anterior cingulate cortex (33), a region significant for MOR BP_{ND} and reward differences in the current study. Thus, as previously suggested (18), it may be an oversimplification to consider the A118G SNP to be either a gain or loss of function SNP, and genotype effects may vary depending on the specific pharmacological challenge.

It should be noted that the absence of differences in MOR BP_{ND} following denicotinzed and nicotine cigarettes (versus controls) contrasts with the only study of MOR BP_{ND} in smokers, which was a small pilot feasibility study (34). The discrepant results are likely attributable to differences in the study populations and designs. For example, the prior study included 6 smokers (all male) compared with 22 smokers of both genders and matched *OPRM1* genotypes (oversampling the */G group) in the current study. Further, the current study was designed to eliminate carryover effects of smoking by scanning smokers on two separate occasions. It will be important in future studies to include sufficient numbers of males and females, as the behavioral effects of this polymorphism may be sex specific (14, 18, 35), and female sex and estradiol alter MOR binding availability (36, 37).

This study has several limitations. BP_{ND} values for nonsmokers in our study are lower than findings reported in some previously published studies (38, 39); however, these values were comparable to other studies (40, 41). These differences in MOR BP_{ND} may be attributable to the oversampling in our study of carriers of the *OPRM1* G allele who have lower BP_{ND} (comprising ~50% of our sample versus 15 20% in the general population). It should also be mentioned that we did not assess plasma nicotine levels nor did we use a smoking topography device to measure puff volumes. Although there may be individual differences in puff volume, this is mitigated by using a standardized puffing procedure (42) and demonstrating significant differences in exhaled CO and subjective reward between sessions. Further, prior studies demonstrated large differences (>0.5 mg) in plasma nicotine levels between the 0.6 mg and 0.05 mg Quest cigarettes, which persist up to 5 h after smoking (43). Lastly, the number of female smokers was insufficient to test for sex heterogeneity in genotype effects or MOR BP_{ND} observed in prior studies (14, 37). However, sex was considered as a covariate in all models and did not predict MOR BP_{ND}. As is the case with any PET receptor binding potential study, it is not possible to disentangle differences in receptor numbers from receptor binding affinity or endogenous opioid tone, a question best answered in preclinical models.

This PET imaging study demonstrates that preclinical evidence for genetic differences in MOR protein translates to in vivo observations of decreased MOR binding availability in human smokers. This is a critical first step toward elucidating the mechanistic basis of previously observed genetic associations with smoking behavior and other drug abuse phenotypes, which have high comorbidity with smoking. This work lays the foundation for future studies to evaluate whether MOR BP_{ND} predicts smoking relapse in treatment seekers. PET imaging of MORs at pretreatment could potentially identify relapse prone smokers, thereby optimizing treatment selection and improving outcomes for this major medical and public health problem.

Materials and Methods

Participants. The study was approved by the University of Pennsylvania Institutional Review Board, the Food and Drug Administration, and the Environmental Health and Radiation Safety Committee. All participants provided written informed consent. Smokers (n = 22) and nonsmokers (n = 20) were recruited through mass media. Smokers were required to report consumption of ≥10 cigarettes per day for at least the past 6 mo and provide a baseline breath carbon monoxide (CO) reading >10 ppm. Nonsmokers were required to report smoking <100 cigarettes/lifetime, no cigarettes in the past year, and provide a CO reading of <5 ppm (CO readings allowed for effects of environmental CO). Individuals with a history of or current neurological or DSM IV Axis I psychiatric or substance disorders (except nicotine dependence), and those taking psychotropic medications (e.g., antidepressants, antipsychotics, anxiolytics, and mood stabilizers) or opioid analgesics were excluded. Due to the influence of female sex hormones on MOR levels (37), postmenopausal women were excluded from the study. Only individuals of European ancestry were included due to ethnic differences in allele frequencies (10).

Procedures. Following preliminary eligibility assessment, participants were assessed for *OPRM1* A118G (rs1799971) genotype using Taqman SNP genotyping assays (Applied Biosystems). Of 51 smokers who were eligible at initial screening, 26 smokers were eligible after *OPRM1* genotype, and 22 smokers completed both their PET scans (12 A/A, 10 */G); 3 smokers were ineligible at their PET scan 1, and 1 participant withdrew before their first PET scan. Of 59 nonsmokers who were eligible at the medical screen, only 21 were eligible on the basis of *OPRM1* genotype and all participants completed their PET scan. One nonsmoker was excluded due to lower than threshold (<3 mCi) radio activity injected during their PET scan, leaving a final sample of 20 nonsmokers (10 A/A, 10 */G).

The final sample of 16 male and six female smokers included 12 A/A, 9 A/G, and 1 G/G *OPRM1* A118G genotype (A/G and G/G genotype groups combined, denoted as */G). These smokers reported a mean age of 31.3 y (SD = 10.7), smoking rate of 17.6 cigarettes/day (SD = 6.3), and Fagerstrom test for nicotine dependence score of 4.68 (SD = 2.01). The final sample of 13 male and seven female nonsmokers included 10 A/A and 10 */G genotypes, with a mean age of 30.9 y (SD = 12.3). There were no significant differences in demographics between A/A and */G groups, between smokers and nonsmokers, or between genotype groups in smoking history (smokers only) (all P values >0.10).

Following 14 h of overnight abstinence (verified by expired CO < 10 ppm), smokers participated in two 60 min [^{11}C]carfentanil PET scans on two separate occasions. Fifteen minutes before each scan, participants smoked a Quest research cigarette (Vector Tobacco). The nicotine cigarette (0.6 mg nicotine) was smoked before scan 1 and the placebo cigarette (denicotinized; 0.05 mg nicotine) was smoked before scan 2. Participants and data analysts were blind to the cigarette type. To standardize exposure, the cigarettes were smoked through a controlled puffing procedure (participants were allowed to take a total of six puffs after lighting the cigarette, each puff was separated by 30 s) (42). The breath expired CO level change from pre to postcigarette was 4.95 (SD = 1.73) after the nicotine cigarette and 3.27 (SD = 1.42) after the denicotinized cigarette (T = 4.71, $P = 1 \times 10^{-4}$). After smoking, participants completed a questionnaire to evaluate the smoking reward they experienced from the cigarette (44). Female participants were scanned during their early follicular phase (2 9 d after menses) to minimize the effects of rising levels of es

trogen on the endogenous opioid system (45). The time interval between the two scans was 1 4 wk (as female participants were scanned during their early follicular phase).

Image Acquisition and Reconstruction. Scans were acquired on a GSO based brain PET scanner, which operates without septa to increase the sensitivity of the instrument (46). Six sequential 10 min long scans were acquired starting immediately after slow bolus injection (over 10 min) of 3 15 mCi [^{11}C]carfentanil, so as not to exceed 0.03 μg/kg. The dose of [^{11}C]carfentanil administered was based on published PET studies (36, 47, 48). The range of specific activity for [^{11}C]carfentanil was 1,500 3,000 mCi/μmol. Upon completion of the emission scan, a transmission scan was acquired using a ^{137}Cs point source and used to perform a segmented attenuation correction. The PET data were reconstructed using an iterative 3D RAMLA algorithm with scatter correction performed using a fully 3D single scatter simulation algorithm (49). The resulting six images consisted of 128 slices of 2 mm slice thickness (2 × 2 mm^2 pixels) and an image matrix size of 128 × 128 pixels).

Single Subject Image Preprocessing. The raw PET data were converted to Neuroimaging Informatics Technology Initiative format and preprocessed using SPM8 (Wellcome Trust Center for Neuroimaging, London, United Kingdom) implemented in Matlab 7.10. The mean image calculated from each subject's six images (0 60 min) was manually reoriented to set the origin to the anterior commissure (AC) with slice position aligned to the AC posterior commissure (PC) line. The reorientation translation parameters were then applied to all six images. The mean image was spatially normalized to the T1 weighted template provided by the Montreal Neurological Institute by means of a least squares approach and 12 parameter spatial transformation followed by estimating nonlinear deformations. Default parameters as defined in SPM8 were used except the bounding box, which was matched to the T1 template. A brain mask in each subject's native space was derived by applying the inverse of the deformation fields to the T1 template. To transform the T1 template in native subject space, deformation fields were computed from the individual normalization parameters using the "Deformations" toolbox in SPM8. Nonbrain areas were removed using this brain mask from all six images.

Regional Time Activity Curve (TAC) Computation. Left and right hemisphere masks were defined for the ACC, AMY, CAU, VST, and THA using the Harvard Oxford probabilistic map (maximal probability threshold: 25%) distributed with FSL software (50). The ventral striatum ROI was composed of the nucleus accumbens and the ventral part of putamen and caudate nucleus using methods described above. The spatially transformed ROIs were then applied to the subject's six images and regional TACs were extracted.

Quantification of MOR Binding (BP_{ND}). MOR binding was estimated using MRTM2 (23) with a fixed value for the tissue to plasma efflux rate constant in the reference region (k_2'). The distribution volume ratio (DVR) is calculated from the ratio of the regression coefficients (23) and the binding potential is given by BP_{ND} = DVR 1. A k_2' value of 0.1237 min ^{1} was assigned on the basis of a previous [^{11}C]carfentanil study (51). The MRTM2 BP_{ND} values calculated for each ROI were exported for further statistical analysis using Stata software.

Statistical Analysis. Because the outcome of interest (MOR BP_{ND} by ROI) is continuous, and observations sometimes included repeated measures (across sessions), we analyzed the data using a multiple linear regression and used the cluster corrected robust covariance matrix to correct SEs (52). We tested the effect of the *OPRM1* genotype (A/A vs. */G) in separate models of MOR BP_{ND} for smokers, adjusted for age, sex, session, and cigarettes per day and for nonsmokers, not adjusted for session. Results reported are from models testing both hemispheres simultaneously using "seemingly unrelated estimation" used to test similarity across hemispheres (53). Additionally, in the smoker models, we used the Wald χ^2 to test for genotype × session interaction. Alpha was adjusted on the basis of 10 ROIs with an average correlation of 0.55, resulting in an adjusted P value of 0.018 (54). On the basis of correlated observations for BP_{ND} in the different ROIs, 22 smokers gave us 80% power to detect a session effect size of 0.664 (correlation adjusted to 0.39) for a one sample test and a genotype (between) effect size of 1.26. This compares favorably to prior MOR binding studies with similar manipulations (24, 55). Secondary analysis examined the Spearman's correlation of MOR BP_{ND} (by ROI) with the continuous smoking reward measure. Significance was obtained using linear regression, with SEs corrected for cluster correlation (tested at P = 0.05).

ACKNOWLEDGMENTS. We thank the following individuals for their contributions to the study: Dr. Richard Freifelder, Dr. Joel Karp, Dr. Alexander Schmitz, and Rahul Poria for [^{11}C]carfentanil synthesis; Dr. Daniel Pryma and Dr. Rodolfo Perini for serving as PET center injectors; and Dr. Janet Reddin and PET center technologists for PET acquisition and preprocessing at the PET center. This research was supported by National Institute on Drug Abuse Grants R21 DA027066 (to C.L. and J.A.B.) and U01 DA020830 (to C.L.), National Cancer Institute Grant P50 CA143187 (to C.L. and J.A.B.), and a grant from the Pennsylvania Department of Health.

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Drug and Alcohol Dependence xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Effects of acute nicotine and alcohol on the rating of attractiveness in social smokers and alcohol drinkers

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ARTICLE INFO

Article history:
Received 28 January 2012
Received in revised form 14 March 2012
Accepted 15 March 2012
Available online xxx

Keywords:
Alcohol
Nicotine
Attractiveness
Reinforcement

ABSTRACT

Background: Nicotine and alcohol are often consumed together. Previous research suggests that both can independently increase the perceived attractiveness of social stimuli, which may be a mechanism that drives continued use. This study examined whether there was an additive effect of nicotine and alcohol on perceived attractiveness of social and environmental stimuli.
Methods: Male and female ($n = 96$) social alcohol consumers and light cigarette smokers (no more than 14 cigarettes per week) were randomized to smoke either a nicotinized or denicotinized cigarette and drink either an alcoholic or non-alcoholic (placebo) beverage. The primary outcome was attractiveness ratings of facial and landscape stimuli. Secondary outcomes were self-report mood and craving.
Results: There was a main effect of drink ($p = .031$) and a trend toward a main effect of cigarette ($p = .057$) with higher ratings of attractiveness after alcohol compared to placebo and after a nicotinized cigarette compared to a denicotinized cigarette. Nicotine and alcohol appeared to work additively on ratings of attractiveness, with the highest ratings in the nicotine/alcohol group. There were no interactions between drink, cigarette and stimulus type.
Conclusions: When co-administered, nicotine and alcohol consumption resulted in the highest perceptions of attractiveness across all stimulus types. This additive effect may be a mechanism by which administration of one drug reinforces use of the other, and which leads to an increased likelihood of habitual consumption and relapse.

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1. Introduction

Nicotine and alcohol use are associated with substantial personal and societal harms. Approximately 4% of the global burden of disease is attributable to alcohol use (Room et al., 2005), and there is growing concern over rates of heavy episodic drinking (binge drinking, particularly in young women who have shown an increase in binge drinking prevalence in recent years; Dantzer et al., 2006; Smith and Foxtrot, 2009). Harmful drinking is not only a risk factor for non-communicable diseases such as liver cirrhosis and cardiovascular disease (O'Flynn, 2011), but acute intoxication is also associated with increases in risky behaviors including aggression and unprotected sex (Chersich and Rees, 2010). Furthermore, the economic cost of binge drinking is substantial. In 2006 alone, binge drinking was estimated to cost the US government around 170.7 billion dollars (Bouchery et al., 2011). Similar concerns exist regarding smoking, which remains the main cause of premature and preventable deaths worldwide, and has been identified as a risk factor in six out of the eight leading causes of global deaths (World Health Organization, 2008). Furthermore, light smoking (i.e., 1–4 cigarettes per day) and mere exposure to smoke has been linked to greater risk of disease and death (Bjartveit and Tverdal, 2005; Oberg et al., 2011).

It is therefore unsurprising that a considerable body of research exists investigating the independent reinforcing effects of nicotine and alcohol. However, nicotine (via smoking) and alcohol are often consumed together, and co-dependency is common (Jacques et al., 2010). Their effects may work additively such that use of one drug reinforces use of the other and thereby promotes a continued cycle of co-administration (Schmitz et al., 2003). In a study of outpatients seeking treatment for concurrent tobacco and alcohol dependence, participants made choices between nicotine and alcohol, and monetary rewards. A combination of alcohol and nicotine was preferred over either substance alone (Schmitz et al., 2003), suggesting a greater relative reinforcement value when both alcohol and nicotine are co-administered. Furthermore, a reciprocal relationship is implicated in studies that identify smoking as a risk factor for alcohol use and alcohol use as a risk factor for smoking (Ait-Daoud et al., 2005; Hurley et al., 2012; Lajtha and Sershen, 2010). Although some biological mechanisms have

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doi:10.1016/j.drugalcdep.2012.03.014

Please cite this article in press as: Attwood, A.S., et al., Effects of acute nicotine and alcohol on the rating of attractiveness in social smokers and alcohol drinkers. Drug Alcohol Depend. (2012), doi:10.1016/j.drugalcdep.2012.03.014

been suggested, the nature of this relationship is likely to consist of multiple components that are not fully understood (Lajtha and Sershen, 2010). Previous research has suggested that both nicotine and alcohol can ameliorate blunting of responses to emotional film clips that occurs during nicotine withdrawal (Dawkins and Powell, 2011), however it remains unclear how alcohol and nicotine may interact in non-withdrawn states. There is a need for greater understanding of the combined effects of nicotine and alcohol, in order to improve cessation interventions and help to prevent relapse.

There is a strong popular belief that alcohol consumption increases the perceived attractiveness of others, an effect known colloquially as the "beer goggles effect," which has received some empirical support. For example, Parker et al. (2008) reported higher ratings of attractiveness of facial stimuli in a group of social drinkers who had consumed 0.4 g/kg alcohol, compared to a group who had consumed placebo. The mechanism by which alcohol exerts this effect is not well understood, but Parker et al. (2008) report that their findings were independent of basic changes in mood or alcohol craving.

Recent data indicates that nicotine may also increase the perceived attractiveness of facial stimuli. Attwood et al. (2009) found higher ratings of attractiveness of male and female faces following a nicotinized cigarette, compared to a denicotinized cigarette. Nicotine is a potent secondary reinforcer that enhances the reinforcing properties of other rewarding stimuli (Chaudhri et al., 2006), via sensitization of central reward systems. As faces are salient social stimuli that are preferentially attended to from an early age (Park et al., 2009; Slater and Quinn, 2001; Slater et al., 1998; Sugita, 2009), it is suggested that nicotine may increase perceived attractiveness by increasing the inherent "reward" value of the faces.

The ability of nicotine to increase the rewarding value or positive affective response to other stimuli has been identified as a factor underlying its habitual use (Caggiula et al., 2009; Donny et al., 2011; Hurley et al., 2012). Therefore, a combined effect of nicotine and alcohol on responses to environmental stimuli may be a mechanism that drives their concurrent use, and examination of this is warranted given our previous findings that both can independently increase attractiveness ratings of facial and environmental stimuli (Attwood et al., 2009; Parker et al., 2008). Therefore, the current study examined the combined effects of nicotine and alcohol on ratings of attractiveness. It is plausible that these effects may work additively to increase perceived attractiveness to a greater extent than when either drug is administered alone. Social smokers and alcohol drinkers were recruited and were randomized to drink either an alcoholic or non-alcoholic beverage, and to smoke either a nicotinized or denicotinized cigarette. We restricted inclusion to light (non-dependent) smokers, who were not expected to experience substantial withdrawal upon abstinence, in order to explicitly test the effects of nicotine, rather than nicotine withdrawal. All participants rated the attractiveness of a series of male and female facial stimuli. Furthermore, to establish whether the effects were specific to faces, an additional stimulus block of landscapes was included. Based on our previous findings (Attwood et al., 2009; Parker et al., 2008), it was hypothesized that there would be main effects of alcohol and nicotine on ratings of attractiveness, and that ratings would be highest in a group of participants who received both alcohol and nicotine.

2. Methods

2.1. Study design

Participants were allocated to one of four groups using a factorial design in which they were asked to drink either an alcoholic or a non-alcoholic beverage and smoke either a nicotinized or denicotinized cigarette. Group allocation was randomized with the constraint that groups contained equal numbers of male and female participants. The experimental design comprised two between-subjects factors of drink (alcohol, placebo) and cigarette (nicotinized, denicotinized), and a within-subjects factor of picture type (faces, landscape). A mixed-model was employed due to potential problems with repeat testing of the attractiveness task. Ratings may alter as participants become more familiar with the images and/or participants may remember previous ratings for an individual and repeat these. A robust randomization procedure was used and baseline tests were conducted to investigate between-subject differences. Ethical approval was granted by the Faculty of Science Research Ethics Committee at the University of Bristol.

2.2. Participants

Ninety-six (50% male) social alcohol drinkers (defined as consuming between 10 and 50 Units/week for males, and between 5 and 35 units/week for females. Note: 1 unit = 8 g ethanol) and light smokers (defined as smoking no more than 14 cigarettes per week and not smoking within 1 h of waking) were recruited from the staff and students of the University of Bristol and from the general population by means of poster and flyer advertisements, and word-of-mouth. Although every participant was required to be a self-reported smoker, there was no stipulated minimum weekly consumption level as many social smokers smoke infrequently at social events, which makes a weekly consumption estimate inappropriate or unreliable. Participants were required to be in good psychological and physical health, aged between 18 and 30 years, heterosexual and not currently taking any psychiatric medication, as verified by self-report. Exclusion criteria included current use of illicit substances (excluding cannabis), a direct family history of alcoholism (defined as parent and/or sibling) and nicotine dependence (defined as a score of 3 or more on the Fagerström Test for Nicotine Dependence (Heatherton et al., 1991). Participants were asked to abstain from drinking alcohol and smoking for 12 h prior to each test session. Participants were reimbursed £5 or awarded course credit, as appropriate, at the end of the study.

2.3. Materials

Cues for the attractiveness task comprised 40 photographic facial stimuli (20 male and 20 female, mean age 21 years) (Attwood et al., 2009; Parker et al., 2008). The facial photographic images were collected using a Canon EOS300D at a focal length of 50 mm under standardized lighting conditions provided by two lateral diffuse portaflash DL1000 studio lights. Images were captured at a resolution of 2048 by 3072 pixels, in full-color. There was a constant background for all images and clothing was masked with a neutral smock. Participants were asked to pose, standing, with a neutral expression and photographed at a distance of approximately 150 cm. The camera lens was at the same horizontal plane as the eyes for each photograph. This procedure provides replicable images of the same individual and provides images that are equivalent to experience of faces in day-to-day interaction (Stephan et al., 2004). All the photographic subjects were University of Bristol undergraduate students, and each individual was the current heterosexual partner of one other individual in the opposite-sex face set. The photographs were taken several years prior to data collection and therefore it is unlikely that any of the participants were familiar with the target individuals. The landscapes comprised 20 images of natural scenes, some of which included buildings, but no people or animals. Images were chosen that ranged from low to high attractiveness (details available on request). Attractiveness for each image was rated on a seven-point scale ranging from very unattractive to very attractive.

The questionnaire measures used included the Fagerström Test of Nicotine Dependence (Heatherton et al., 1991), the Eysenck Personality Inventory – Revised (Eysenck and Eysenck, 1991), the Alcohol Use Disorders Identification Test (Saunders et al., 1993), the Positive and Negative Affect Schedule (Watson et al., 1988), the Questionnaire of Smoking Urges (Tiffany and Drobes, 1991), the Alcohol Urges Questionnaire (Bohn et al., 1995) and visual analog scales of mood, anxiety and craving, comprising the items "happy," "drowsy," "depressed," "anxious," "energetic," "irritable," and "intoxicated" rated on a 100 mm scale from "Not At All" to "Extremely."

2.4. Procedure

Experimental sessions lasted approximately 50 min and all testing took place between 12:00 and 18:00 h. Upon arrival, participants were given an opportunity to read the information sheet and ask questions before providing informed consent. They were informed that during the study they would be required to drink a lime/tonic drink that may or may not contain alcohol and smoke a cigarette that may or may not contain nicotine. Participants were screened to ensure that they met the inclusion criteria (including completion of the Fagerström Test of Nicotine Dependence), and provided a breath sample to test breath alcohol (PT400, UK Breathalyzers) and carbon monoxide (Micro Smokerlyzer, Bedfont UK) levels. Participants were then weighed and the weight was relayed to an experimental collaborator who made up the drink and delivered it to the researcher, such that both the participant and researcher were blind to the alcohol content of the drink.

While the drink was being prepared, participants completed baseline measures of craving and mood (Questionnaire of Smoking Urges, Alcohol Urges Questionnaire, Positive and Negative Affect Schedule, visual analog scales). Participants were randomized to receive either 0.4 g/kg of alcohol, using vodka at 37.5% alcohol, with one-part vodka to three parts tonic water, or a placebo consisting of an equal total

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volume of tonic water. All drinks were chilled and flavored with lime cordial. Previous taste-test pilot studies in our laboratory have demonstrated that participants identify this drink as alcoholic at chance level, when measured at the time of consumption. Participants were given 10 min to consume the drink and a further 10 min to sit quietly to allow for absorption, during which time they completed trait measures of drinking behavior and personality (Alcohol Use Disorders Identification Test, Eysenck Personality Inventory – Revised).

After this absorption period, participants were taken to a specially ventilated smoking cubicle that contained a computer, table, chair and ashtray. Once they were seated comfortably that were given a cigarette and asked to light it and take a puff. Half of the participants received a nicotinized (0.6 mg nicotine) and half of the participants received a denicotinized (0.005 mg nicotine) cigarette (balanced across alcohol groups). Cigarettes were commercially available reduced nicotine Quest™ cigarettes (Vector Tobacco Ltd., Durham NC) that had been labeled independently by a third party, so that cigarette administration was double-blind. Once participants had taken a puff of the cigarette, they began the computer task. Photographic stimuli were presented in a blocked format (male faces, female faces, landscapes), with block order randomized across participants. Each individual image appeared on screen for 800 ms. Participants were then required to rate each image for attractiveness, using a 7-point Likert scale anchored at "very unattractive" and "very attractive". Responses were entered using numeric keys on the testing computer. The presentation of stimuli was controlled by the E-Prime (v.1.2) software package (Psychology Software Tools Inc., Pittsburgh, PA).

On completion of the attractiveness rating task, participants completed self-report measures of craving and mood, and were asked whether they believed that their drink had contained alcohol and whether their cigarette had contained nicotine. Participants were then debriefed as to the purpose of the study and reimbursed as appropriate.

2.5. Statistical analysis

Data were analyzed using $2 \times 2 \times 2$ mixed model ANOVAs with drink (alcohol, placebo) and cigarette (nicotinized, denicotinized) as between-subjects factors. For task data, picture type (faces, landscapes) was a within-subjects factor, and for subjective mood (Positive and Negative Affect Schedule, visual analog scales) and craving (Alcohol Urges Questionnaire, Questionnaire of Smoking Urges) data time (baseline, post-session) was a within-subjects factor. To correct for multiple comparisons in visual analog scale analyses, a Bonferroni adjusted alpha of .007 was used.

Where significant drug-related effects were observed, we conducted additional 4×2 mixed model ANOVAs with group (placebo nicotine/placebo alcohol; placebo nicotine/alcohol; nicotine/placebo alcohol; nicotine/alcohol) as a between-subjects factor, in order to examine simple group effects. These included a within-subjects factor of picture type (faces, landscapes) for attractiveness data and time (baseline, post-session) for subjective data. In order to control for inflated Type I error in post hoc tests of group, Tukey's HSD test was used. Additional secondary analyses were conducted to assess participant sex by target sex interactions across drug administration, using data from the male and female picture sets only. This comprised a $2 \times 2 \times 2 \times 2$ mixed model ANOVA with drink (alcohol, placebo), cigarette (nicotine, placebo) and participant sex (male, female) as between-subjects factors and target sex (male, female) as a within-subjects factor.

Our sample size was sufficient to detect a difference of 0.3 points on ratings of attractiveness (based on previous data indicating a mean of 4.00 and a standard deviation of 0.5), with 80% power at an alpha level of 0.05.

Exact p-values are reported throughout. All analyses were conducted using SPSS version 18.

3. Results

3.1. Participant demographics

Participants ($n = 96$; 50% male) were on average aged 22 years ($SD = 3$, range 18–30), weighed 68 kg ($SD = 11$, range 50–94), had a mean Alcohol Use Disorders Identification Test score of 13 ($SD = 5$, range 2–30), and a mean Fagerström Test of Nicotine Dependence score of 0.06 ($SD = 0.3$, range 0–2). A series of one-way ANOVAs indicated no significant differences between groups on age ($p = .52$), weight ($p = .51$), Fagerström Test of Nicotine Dependence score ($p = .75$), Alcohol Use Disorders Identification Test score ($p = .57$), Eysenck Personality Questionnaire – Extraversion ($p = .70$), Eysenck Personality Questionnaire – Neuroticism ($p = .44$) or Eysenck Personality Questionnaire – Psychoticism ($p = .36$). Participant characteristics are summarized in Table 1.

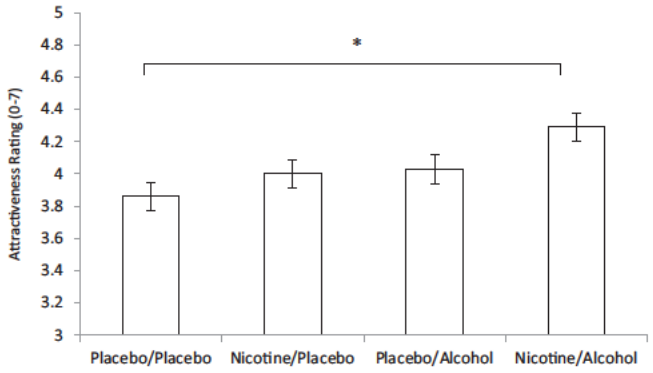


Fig. 1. Mean (±SEM) attractiveness rating (1–7) across all stimulus conditions in the four drug groups. *$p < .05$.

3.2. Attractiveness ratings

There was a main effect of picture type ($F[1,92] = 137.8$, $p < .001$, partial $\eta^2 = .60$) with landscapes being rated as more attractive ($M = 4.5$, $SD = 0.7$) than faces ($M = 3.6$, $SD = 0.6$). There was also a main effect of drink ($F[1,92] = 4.78$, $p = .031$, partial $\eta^2 = .05$) with higher ratings of attractiveness after alcohol ($M = 4.2$, $SD = 0.6$) compared to placebo ($M = 3.9$, $SD = 0.6$) and there was also a trend toward a main effect of cigarette ($F[1,92] = 3.72$, $p = .057$, partial $\eta^2 = .04$) with higher ratings of attractiveness after a nicotinized cigarettes ($M = 4.1$, $SD = 0.6$) compared to a denicotinized cigarette ($M = 3.9$, $SD = 0.7$). There were no other main effects or interactions ($ps > .52$). We conducted additional analyses using a 4×2 mixed model ANOVA with drug group (placebo/placebo, nicotine/placebo, placebo/alcohol, nicotine/alcohol) as a between-subjects factor and picture type (faces, landscapes) as a within-subject. In addition, to a main effect of picture type (see above), there was also a main effect of drug group ($F[1,92] = 2.93$, $p = .038$, partial $\eta^2 = .09$), with lowest ratings of attractiveness in the placebo/placebo group and highest in the nicotine/alcohol group, and with intermediary ratings in the nicotine/placebo and placebo alcohol groups (see Fig. 1). Tukey's HSD comparisons indicated that the difference between nicotine/alcohol and placebo/placebo groups reached statistical significance ($p = .023$). There was no evidence of a picture type by group interaction ($p = .93$).

3.2.1. Secondary analysis of gender effects. The $2 \times 2 \times 2 \times 2$ mixed model ANOVA investigating effects of participant sex and target sex across drug groups indicated a main effect of target sex ($F[1,88] = 110.16$, $p < .001$, partial $\eta^2 = .56$), with higher ratings of attractiveness for female ($M = 4.0$, $SD = 0.7$) compared to male ($M = 3.3$, $SD = 0.7$) targets. All other main effects and interactions involving target and participant sex were non-significant ($ps > .21$).

3.3. Subjective data

3.3.1. Positive and negative affect schedule. Due to failure to complete the questionnaire, data from two participants were missing from positive effect analyses and from one participant for negative effect analyses. There were main effects of time for positive ($F[1,90] = 21.46$, $p < .001$, partial $\eta^2 = .14$) and negative ($F[1,92] = 15.8$, $p < .001$, partial $\eta^2 = .15$) affect, with decreases in both positive and negative affect across the session. For positive affect there was a main effect of nicotine with higher ratings of positive affect in nicotine groups compared to placebo nicotine groups ($F[1,90] = 4.97$, $p = .028$, partial $\eta^2 = .05$). There was also a significant time × nicotine × alcohol interaction ($F[1,90] = 7.41$, $p = .008$, partial $\eta^2 = .08$) for positive affect. Post hoc ANOVAs revealed a time × nicotine interaction for alcohol ($F[1,45] = 5.65$, $p = .022$,

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Table 1
Characteristics of participants.

	Placebo/placebo $n=24$	Nicotine/placebo $n=24$	Placebo/alcohol $n=24$	Nicotine/alcohol $n=24$
Age	21(2)	22(4)	22(3)	21(3)
Weight	69(11)	69(12)	67(10)	65(10)
FTND	0.08 (0.3)	0.08 (0.4)	0(0)	0.8 (0.4)
AUDIT	14(7)	14(6)	14(5)	12(4)
EPQ-E	18(3)	18(4)	18(3)	17(4)
EPQ-N	12(6)	10(6)	13(6)	13(5)
EPQ-P	9(3)	7(4)	9(3)	8(3)

Values represent mean (standard deviation). *Abbreviations*: EPQ-E = Extraversion; EPQ-N = Neuroticism; EPQ-P = Psychoticism.

partial $\eta^2 = .11$) but not placebo alcohol conditions ($F[1,45] = 2.05$, $p = .16$, partial $\eta^2 < .04$). When alcohol was administered alone there was a decrease in positive affect over time, which did not occur when alcohol and nicotine were co-administered (see Fig. 2). There were no other significant main effects or interactions (*ps* > .19).

3.3.2. Visual Analog Scales. There were main effects of time on ratings of energetic ($F[1,92] = 9.07$, $p = .003$, partial $\eta^2 = .09$) and intoxicated ($F[1,92] = 206.14$, $p < .001$, partial $\eta^2 = .69$), with decreases in energetic ratings over time and increases in intoxication. There was a main effect of alcohol for ratings of intoxication ($F[1,92] = 12.55$, $p = .001$, partial $\eta^2 = .12$) which was subsumed under a significant time × alcohol interaction ($F[1,92] = 20.32$, $p < .001$, partial $\eta^2 = .18$). Ratings of intoxication increased after both alcohol ($t = -13.49$, df = 47, $p < .001$) and placebo alcohol ($t = -6.73$, df = 47, $p < .001$); however, the magnitude of this increase was greater after alcohol compared to placebo. We also conducted a 4 × 2 mixed model ANOVA for intoxication ratings with drug group (placebo/placebo, nicotine/placebo, placebo/alcohol, nicotine/alcohol) as a between-subjects factor and time (baseline, post-session) as a within-subjects factor. Consistent with the initial analysis, there was a main effect of drug group ($F[1,92] = 5.03$, $p = .003$, partial $\eta^2 = .14$) and a significant time × group interaction ($F[3,92] = 8.22$, $p < .001$, partial $\eta^2 = .21$). There was a significant increase in self-reported intoxication over time in all groups (*ps* < .001), however increases were greater after alcohol compared to no alcohol and greatest in the group who received both nicotine and alcohol (see Fig. 3).

3.3.3. Questionnaire of Smoking Urges. There was a main effect of alcohol ($F[1,92] = 6.52$, $p = .012$, partial $\eta^2 = .06$), with higher craving scores in individuals who received alcohol ($M = 24$, $SD = 9$) compared to those who received placebo alcohol ($M = 20$, $SD = 8$). There were no other main effects or interactions (*ps* > .23).

3.3.4. Alcohol Urges Questionnaire. There were no significant main effects or interactions (*ps* > .07).

3.4. Awareness check

At the end of the session participants were asked to indicate whether they thought that their drink had contained alcohol, and whether their cigarette had contained nicotine. This was not done directly after drink/cigarette administration, in order to avoid priming effects. Participants were accurately able to judge drink content in 77% of cases, and cigarette content in 68% of cases, both of which differed significantly from chance: (alcohol χ^2 (1, $n = 96$) = 13.77), $p < .001$; nicotine (χ^2 (1, $n = 96$) = 30.79, $p < .001$).

4. Discussion

Our results demonstrated a significant main effect of drink and a trend main effect of cigarette, with higher ratings of attractiveness after alcohol compared to placebo alcohol, and after a nicotinized compared to a denicotinized (placebo) cigarette. Highest ratings were observed in individuals who had received both nicotine and alcohol but there was no alcohol by nicotine interaction, suggesting an additive, rather than multiplicative effect. This study also demonstrated that these effects were not specific to facial stimuli. The ability of combined nicotine and alcohol to enhance perceived attractiveness of social and environmental stimuli may be a factor that increases the likelihood of developing a habitual pattern of use and dependence.

There was evidence of decreases in positive affect over time in all individuals except those who had received both nicotine and alcohol. However, mood enhancement is unlikely to be the primary mechanism driving the differences in attractiveness reported here, as we did not see main effects of drink or cigarette on positive affect, consistent with the effects observed on attractiveness ratings. Furthermore, previous studies that have administered

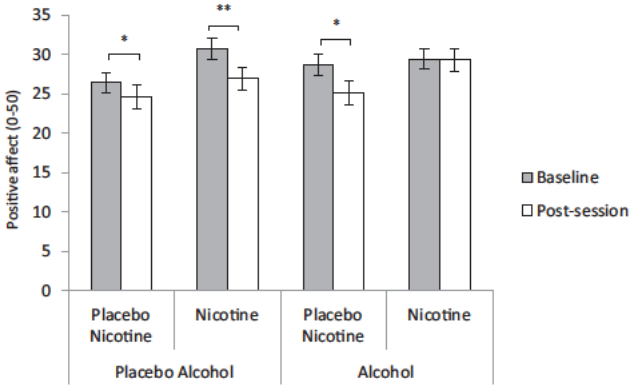


Fig. 2. Mean (±SEM) ratings of positive affect (0–50) at baseline and post-session in alcohol and nicotine groups. *$p < .01$, **$p < .1$.

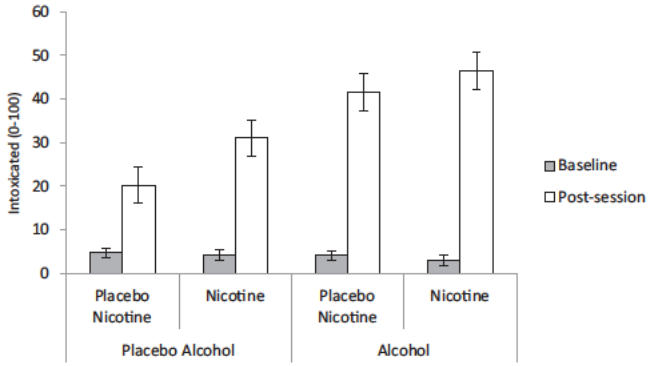


Fig. 3. Mean (±SEM) ratings of visual analog scale intoxication (0–100) at baseline and post-session in the four drug groups.

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alcohol and nicotine alone have found drug-related increases in ratings of attractiveness without corresponding changes in mood (Attwood et al., 2009; Parker et al., 2008). Not unexpectedly, greater increases in subjective intoxication occurred after alcohol compared to placebo. However, significant increases after the non-alcoholic drink over time suggest that this placebo drink was effective in inducing alcohol-related expectancies.

Secondary analyses revealed no effects of participant or target sex on ratings of attractiveness. Due to the addition of a between-subjects factor here (participant sex), there may have been a lack of power to identify small effects. Observation of the means did not reveal any discernable group differences, tentatively suggesting that this null finding would not change with a greater sample size, although this cannot be ruled out. The effect of alcohol on attractiveness is anecdotally considered to be specific to members of the opposite sex. Jones and colleagues (2003) conducted a quasi-experimental study that did not directly administer alcohol but approached bar and restaurant patrons on a university campus, who had either consumed alcohol or not, and found higher ratings of attractiveness in alcohol consumers that were specific to the opposite sex. In contrast, and similar to the current study, Parker et al. (2008) found effects of alcohol across male and female targets in a laboratory study that experimentally manipulated alcohol dose. This suggests that the effects of alcohol on perceived attractiveness may be mediated by context, with sex effects becoming apparent in situations where interactions with the opposite sex are typical. Future laboratory studies could begin to examine this possibility using social manipulations that prime individuals to think about valid contexts or opposite sex exchanges.

Participants were asked to abstain overnight from both nicotine and alcohol prior to the study session. Therefore, it is plausible that the effects observed may have been due to reversal of a withdrawal syndrome, whereby negative physiological and subjective states associated with withdrawal induced relative low ratings of attractiveness akin to the withdrawal-related emotional blunting responses reported by Dawkins and Powell (2011). The implications of this would be that the effects reported here may not generalize beyond abstinent individuals who are experiencing drug-related withdrawal. To reduce the likely impact of withdrawal, we selectively recruited light smokers and alcohol drinkers who were unlikely to experience withdrawal during acute abstinence. In support, our participants self-reported a lack of alcohol dependence and showed no nicotine dependence on the Fagerström Test of Nicotine Dependence, and all reported not smoking within an hour of waking, which is one of the most reliable methods of assessing smoking dependence.

Differences were observed in ratings of attractiveness across stimulus types. Landscapes were rated as more attractive than faces, and female faces were rated as more attractive than male faces. These differences were not unexpected as although a range of low and high stimuli were chosen *within* each stimulus set, the mean level of attractiveness *across* stimulus sets were not matched, due to a limited number of photographs available. Therefore, we are restricted in the ability to make direct comparisons across stimulus types; however, our interest was related to how these interact with drug administration rather than absolute differences per se.

There are some limitations to the present study which should be considered when interpreting these results. First, participants consumed the drink and smoked the cigarette alone in a laboratory environment. Although this setting lacks ecological validity, we argue that the effects are likely to be stronger in a naturalistic environment in which there may be priming effects in a context associated with alcohol consumption and intoxication, and where there is perceived availability of sexual partners. It is less clear, however, to what extent the findings would change qualitatively (e.g., become more sensitive to influences of sex – see above) in a natural setting or would be influenced by additional social factors such as the presence of others or individual differences such as drug-related expectancies (Friedman et al., 2005). These are important avenues for future research.

In addition, we administered a single dose of nicotine and alcohol in the current study which is not typical of the usual pattern of repeated smoking and drinking in a social setting. Again we argue that the effects may strengthen with repeated doses, but may differ at particularly high doses and during the descending limb of the blood alcohol curve.

It is also noteworthy that our attractiveness ratings were only completed once, at the time of test. Therefore, we cannot rule out that the findings were due to random individual differences. However, it was preferable that the images were unfamiliar at the time of test and therefore we did not include baseline ratings of attractiveness. To help reduce the influence of random individual differences, we employed a robust randomization procedure, and compared groups on relevant baseline measures including personality and dependence. No group differences were found on any of the parameters measured.

Finally, individuals were able to correctly identify the content of their cigarette and drink to a degree greater than chance, and therefore effects of expectancy cannot be ruled out. Attempts were made to disguise the content of the drinks with the addition of lime and tonic and by chilling the drinks prior to serving. Pilot work from our laboratory has shown that at the time of consumption, individuals are unable to identify the content of the drink above chance, which indicates that the drinks are well match on their perceptual properties. To avoid drawing attention to drink/cigarette content, participants were not asked to identify what they thought they had received at the time of consumption. Instead they were asked at the end of the study session, approximately 20 min post-drink and 10 min post cigarette, when the post-consumption effects of the drink and cigarette had been experienced. This is a common limitation in psychopharmacological studies that administer stimulating and/or intoxicating doses of a drug to regular users. Future research could examine the role of drug expectancy using balanced-placebo designs for nicotine and alcohol separately, although using this design in a study administering both drugs is more difficult due to the high number of conditions. However, even in these studies that are explicitly designed to differentiate the effects of expectancy from the pharmacological effects of a drug, this limitation is acknowledged (particularly with alcohol) (Bushman and Cooper, 1990; Seto and Barbaree, 1995). Increases in intoxication were reported in participants who had received the placebo drink, implying that some degree of alcohol expectancy had been induced in this group. On the basis of this limitation, we cannot rule out that the effects observed are due to expectancy effects. We suggest, however, that this does not invalidate the findings as these effects will still operate in the real world, but simply raises question regarding the mechanism underlying these effects. To further understand the impact of placebos, future studies should replicate this study design incorporating a no-drug control group that does not smoke or drink.

In sum, nicotine and alcohol consumption significantly increase ratings of perceived attractiveness compared to placebo, and this effect appears to be additive as evinced by highest ratings when nicotine and alcohol were co-administered. This suggests that co-administration of these drugs enhances the pleasantness of an individual's social and environmental surroundings. This may be a mechanism that drives continued use or potentiates relapse during abstinence of one or both drugs, particularly in situations where the drugs are commonly taken together.

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Disclosures

Role of funding source

This study received no external funding.

Contributors

MRM, ASA and IPV developed the idea and designed the study. ASA and CG wrote the protocol and collected the data. ASA conducted the analysis and wrote the first draft of the manuscript and MRM, IPV and CG contributed to the final draft of the manuscript. All authors have reviewed and approved the final manuscript.

Conflicts of interest

ASA is partially employed by a grant funded by Pfizer Inc. and has received additional funding from Pfizer for conference attendance. MRM has received grant funding from the AERC, the European Research Advisory Board, and Pfizer Ltd., and has received consulting fees from Servier.

Acknowledgements

MRM and ASA are members of the UK Center for Tobacco Control Studies, a U.K. Clinical Research Collaboration Public Health Research Center of Excellence. Funding from the Economic and Social Research Council, the British Heart Foundation, Cancer Research UK, the Department of Health, and the Medical Research Council, under the auspices of the UK Clinical Research Collaboration, is gratefully acknowledged. The authors are grateful to Viviane Rostant for her assistance with drug administration. This study received no external funding.

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Please cite this article in press as: Attwood, A.S., et al., Effects of acute nicotine and alcohol on the rating of attractiveness in social smokers and alcohol drinkers. Drug Alcohol Depend. (2012), doi:10.1016/j.drugalcdep.2012.03.014

Brief Report

Smoking Withdrawal in Smokers With and Without Posttraumatic Stress Disorder

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Received December 7, 2010; accepted June 7, 2011

Abstract

Introduction: Previous research on smoking withdrawal in posttraumatic stress disorder (PTSD) has been limited by the use of retrospective and observational methods and has lacked repeated assessments on the first day of abstinence and evaluation of the conditioned effects of smoking.

Methods: Smokers with (n = 17; 59% female) and without (n = 30; 17% female) PTSD completed 3 randomly ordered experimental sessions using a 2 (group: PTSD vs. non-PTSD) × 3 (smoking condition: usual brand vs. nicotine free vs. no smoking) design. Before the smoking manipulation, participants completed self-report measures of smoking urges and withdrawal, followed by withdrawal assessment after the smoking manipulation.

Results: Compared with smokers without PTSD, smokers with PTSD exhibited higher craving ($\chi^2_1 = 16.60$, $p < .001$) and habit withdrawal ($\chi^2_1 = 10.38$, $p = .001$) following overnight abstinence. PTSD smokers also exhibited worsening negative affect throughout the morning when not smoking a cigarette ($\chi^2_1 = 11.30$, $p = .004$). After smoking, smokers with PTSD reported diminished relief from craving ($\chi^2_1 = 6.49$, $p = .011$), negative affect ($\chi^2_1 = 4.51$, $p = .034$), arousal ($\chi^2_1 = 6.46$, $p = .011$), and habit withdrawal ($\chi^2_1 = 7.22$, $p = .007$), relative to smokers without PTSD.

Conclusions: Results of this preliminary investigation suggested that after overnight abstinence, PTSD smokers experienced worse withdrawal symptoms and greater urges to smoke for both positive and negative reinforcement. Research on smoking withdrawal early in the course of smoking abstinence in PTSD could inform interventions targeting abstinence early in the quit attempt.

Introduction

Individuals with posttraumatic stress disorder (PTSD) are twice as likely to smoke cigarettes and are among the most recalcitrant of smokers to smoking cessation interventions (Lasser et al., 2000; Piper, Cook, Schlam, Jorenby, & Baker, 2010). Although PTSD smokers frequently lapse early in the course of smoking abstinence (Zvolensky et al., 2008), almost no data are available on withdrawal symptoms during the first 24 hr of smoking abstinence. Examination of early withdrawal symptoms is needed to inform models of smoking lapse and relapse in PTSD (Ziedonis et al., 2008).

While results are mixed, studies examining retrospective report suggest that PTSD is associated with increased smoking withdrawal symptoms (Breslau, Kilbey, & Andreski, 1992; Marshall et al., 2008; Weinberger, Maciejewski, McKee, Reutenauer, & Mazure, 2009). These studies are limited, however, by recall bias. Limited prospective evidence suggests that PTSD smokers have elevated total withdrawal symptoms in the first 24 hr of abstinence (Feldner, Vujanovic, Gibson, & Zvolensky, 2008; Zvolensky et al., 2008). There is a need for experimental studies evaluating changes in withdrawal across repeated assessments during the first day of smoking withdrawal.

Increased withdrawal symptoms early in the course of smoking abstinence are significant, especially viewed from the perspective of the affective processing model of negative reinforcement (Baker, Piper, McCarthy, Majeskie, & Fiore, 2004). This model stresses the critical role of negative affect and the interoceptive cues that precede negative affect in smoking lapse and relapse. To address the limitations associated with previous studies in PTSD and smoking withdrawal, we conducted a prospective experimental study in smokers with and without PTSD. This study explored associations of PTSD, nicotine, and conditioned effects of smoking on nicotine withdrawal symptoms. Conditioned effects refer to reinforcing stimuli associated with nicotine administration, including the smoke aroma, irritant reactions in the mouth and throat, and olfactory cues. Evidence suggests that smoking behavior is driven in part by the conditioned effects of smoking (Rose & Levin, 1991), so conditioned effects could also influence smoking withdrawal.

doi: 10.1093/ntr/ntr142
Advance Access published on October 24, 2011
Published by Oxford University Press on behalf of the Society for Research on Nicotine and Tobacco 2011.

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Methods

Participants and Procedures

Data from this preliminary investigation are from a larger study investigating acoustic startle in smokers with and without PTSD (Calhoun et al., 2011). Eligible participants were generally healthy, were not seeking treatment for nicotine dependence, and smoked at least 15 cigarettes/day with afternoon expired carbon monoxide (CO) ≥15 ppm. Participants were excluded for major unstable medical problems using noncigarette forms of nicotine, schizophrenia, current manic episode, lifetime but not current PTSD, current major depressive disorder, current substance abuse/dependence, current benzodiazepine use, or hearing deficits in the 500–3,000 Hz range.

Participants were recruited via flyers and clinician referrals from local outpatient clinics. Psychiatric diagnoses were based on the Clinician-Administered PTSD Scale (Blake et al., 1995) and the Structured Clinical Interview for *DSM-IV* Disorders (First, Spitzer, Williams, & Gibbon, 2002); average Fleiss' (1981) kappa across nine raters for diagnoses was .95. Participants completed three randomly ordered experimental sessions using a 2 (group: PTSD [n = 17] vs. non-PTSD [n = 30]) × 2 (script: trauma script vs. neutral script) × 3 (smoking condition: usual brand vs. nicotine free vs. no smoking) crossover design. Only neutral script sessions were evaluated in the current report.

Participants were asked to refrain from cigarette, alcohol, and marijuana use the night before testing but maintain their usual caffeine consumption. At the laboratory visit, breath samples confirmed abstinence from smoking based on a previously validated formula (Rose & Behm, 2004) that takes into account baseline CO levels. Participants completed self-report measures, then were offered a small breakfast, and completed startle procedures before smoking their first cigarette of the day approximately 40 min into the session. In the nicotinized cigarette condition, participants smoked their choice of cigarette brand. They had the option to continue to smoke to maintain satiety but rarely did so because sessions ended after 60 min. After reading neutral scripts and completing startle and prepulse inhibition procedures, participants completed self-report measures to end the session. In the denicotinized cigarette condition, participants smoked cigarettes with only trace amounts of nicotine (Quest 3; <.05 mg).

Measures

Shiffman/Jarvik Withdrawal Scale

This 32-item measure assesses the following six common symptoms of nicotine withdrawal: craving (e.g., "Do you have an urge to smoking a cigarette"), negative affect (e.g., "Do you feel tense"), arousal (e.g., "Do you feel wide awake"), somatic (e.g., "Is your heart beating faster than usual"), appetite (e.g., "Do you feel hungrier than usual"), and habit withdrawal (e.g., "Do you miss having something to do with your hands"; Shiffman & Jarvik, 1976). Because participants completed this measure twice during the session, instructions read, "Please fill in the bubble for each question that most accurately reflects how you feel Right Now." The Shiffman/Jarvik Withdrawal Scale (SJWS) has demonstrated the expected decrease in withdrawal symptoms across successive days of abstinence (Shiffman & Jarvik, 1976) and has shown consistent change scores upon abstinence for repeated abstinence periods (Hughes, Hatsukami, Pickens, & Svikis, 1984). Internal consistency in this sample was good (Cronbach's alpha = .82, .84, and .86 at baseline, presmoking, and postsmoking assessments, respectively).

Brief Questionnaire of Smoking Urges

This 10-item measure assesses two subscales of smoking urges. On Factor 1, a higher score indicates a greater urge to smoke in anticipation of pleasure from smoking. A higher score on Factor 2 indicates a greater urge to smoke for relief from withdrawal or negative affect (Tiffany & Drobes, 1991). Psychometric investigation has supported the factor structure and internal consistency in laboratory and clinical settings (Cox, Tiffany, & Christen, 2001). The current sample had good internal consistency (Cronbach's alpha = .92, .94 across presmoking and postsmoking assessments, respectively).

Smoking History and Nicotine Dependence Measures

General smoking history and nicotine dependence were measured at a baseline visit with a demographic and background questionnaire and the Fagerström Test for Nicotine Dependence (FTND; Heatherton, Kozlowski, Frecker, & Fagerström, 1991).

Data Analysis Plan

Generalized estimating equations (GEE) were used to model smoking withdrawal symptoms and symptom change with one between-groups factor, PTSD status (PTSD vs. non-PTSD), one within-subjects factor, smoking condition (nicotinized cigarette vs. denicotinized vs. no smoking), and a PTSD × Smoking Condition interaction. Each subscale on the SJWS was modeled separately. Analyses controlled for nicotine dependence at study entry, and analyses of postsmoking withdrawal symptoms controlled for presmoking withdrawal symptoms.

To test carryover effects of the sequence of smoking conditions on postsmoking withdrawal measures, we used procedures described by Senn (2002) to fit mixed effect linear models, with participants modeled as random effects. These models yielded no evidence of differential carryover of laboratory sessions between different smoking conditions.

Results

Demographic and Baseline Smoking Intensity Measures by Group

Demographic characteristics are summarized in Table 1. The majority of the sample was male (68%) and unmarried (79%), of minority racial background (81%), with a mean age of 43 and mean education of 12 years. The PTSD group had a higher proportion of women than the non-PTSD group (58% vs. 17%, respectively; $\chi^2_1 = 8.87$, $p = .003$). At baseline, neither PTSD diagnosis nor PTSD symptom severity (as measured by the clinician-administered PTSD scale and Davidson Trauma Scale) were significantly associated with smoking measures, including CO level, FTND, and cigarettes per day.

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Table 1. Participant Demographic, Current Diagnostic, and Smoking Characteristics

Variable	PTSD ($N = 17$)	Non-PTSD ($N = 30$)
Age, *M* (*SD*)	45.82 (9.53)	42.53 (8.72)
Education, *M* (*SD*)	11.29 (1.93)	12.63 (2.53)
Sex (% female)	59% (10)*	17% (5)*
Race		
% Black	67% (12)	83% (25)
% Minority	71% (12)	87% (26)
% Married	44% (7)	33% (10)
% Veteran	29% (5)	37% (11)
PTSD-DTS	57.82 (33.36)*	14.83 (17.90)*
Months since trauma	212.15 (186.94)	183.23 (150.61)
CO level at screen	23.29 (7.67)	25.67 (12.75)
FTND	6.53 (1.46)	6.13 (1.98)
Trauma types		
None	0	4
Diagnosed with illness	2	4
Death/illness of loved one	3	6
Childhood sexual abuse	5	3
Childhood physical abuse	0	1
Combat-related traumas	2	1
Adult physical violence	3	7
Adult sexual assault	1	0
Motor vehicle accident	1	4
Current panic disorder	6% (1)	3% (1)
Current obsessive–compulsive disorder	12% (2)	0%
Current phobic disorder	35% (6)	3% (1)
Any non-PTSD anxiety disorder	47% (8)	7% (2)

Note. DTS = Davidson Trauma Scale; FTND = Fagerström Test for Nicotine Dependence; PTSD = posttraumatic stress disorder.
*$p < .05$.

Withdrawal and Smoking Expectancies After Overnight Abstinence by PTSD Group

Withdrawal symptoms are listed by PTSD group and smoking condition in Table 2. GEE analyses of presmoking symptoms revealed that, relative to the non-PTSD group, the PTSD group was higher in craving ($\chi^2_1 = 16.60$, partial $\eta^2 = .179$, $p < .001$) and habit withdrawal ($\chi^2_1 = 10.38$, partial $\eta^2 = .124$, $p = .001$) following overnight abstinence. In addition, the PTSD group was higher in urge to smoke in anticipation of pleasure ($\chi^2_1 = 17.47$, $p < .001$) and to relieve withdrawal/negative affect ($\chi^2_1 = 6.09$, $p = .014$) on the "Brief Questionnaire of Smoking Urges."

Change in Symptoms as a Function of PTSD and Smoking Condition

Main effects of PTSD and smoking condition on the change in withdrawal symptoms throughout the session are presented in Table 2. After the smoking manipulation, the PTSD group had significantly less relief from craving ($\chi^2_1 = 6.49$; $p = .011$) compared with the non-PTSD group. Smoking condition also had a significant effect on craving ($\chi^2_2 = 51.57$; $p < .001$), such that nicotinized cigarettes were associated with a larger decrease in craving than denicotinized cigarettes ($\chi^2_1 = 35.07$; $p < .001$) or the no-smoking condition ($\chi^2_1 = 51.56$; $p < .001$). Craving was significantly reduced by denicotinized cigarettes compared with the no-smoking condition ($\chi^2_1 = 5.82$; $p = .016$).

Analysis of change in negative affect revealed an interaction between PTSD and smoking ($\chi^2_2 = 11.30$; $p = .004$). Follow-up contrasts revealed no statistically significant differences between the PTSD group and the non-PTSD comparison group in the nicotinized or denicotinized conditions. However, in the no-smoking condition, the non-PTSD group exhibited relatively stable levels of negative affect throughout the session, while the PTSD group reported increasing negative affect ($\chi^2_1 = 7.51$; $p = .006$).

Across smoking conditions, PTSD smokers reported greater reduction in arousal symptoms ($\chi^2_1 = 6.46$; $p = .011$) and less reduction in habit withdrawal ($\chi^2_1 = 7.22$; $p = .007$). As expected, there was a main effect of smoking condition on habit withdrawal ($\chi^2_2 = 18.79$; $p < .001$), with a significantly greater reduction following nicotinized cigarettes, relative to denicotinized cigarettes ($\chi^2_1 = 9.23$; $p = .002$) or no-smoking condition ($\chi^2_1 = 18.78$; $p < .001$). Habit withdrawal also reduced more following denicotinized cigarettes, relative to no smoking ($\chi^2_1 = 5.84$; $p = .016$).

Discussion

The results of this experimental study of smoking withdrawal symptoms following overnight abstinence extend findings from previous research documenting more severe smoking withdrawal in PTSD. This report demonstrates worse withdrawal across repeated assessments during the morning following overnight abstinence. In addition, there was no statistically significant between-group difference in baseline nicotine dependence. This unintended design feature reduced the influence of this confound noted in previous reports of smoking withdrawal in PTSD (Feldner et al., 2008). The effects of smoking conditions on withdrawal were generally large, despite the short time interval (approximately 1 hr) between the two measurements of withdrawal.

An interaction of PTSD and smoking suggested that while smokers without PTSD experienced a steady level of negative affect in the first morning of smoking abstinence, smokers with PTSD experienced increasing levels of negative affect. It is noteworthy that negative affect is central to models of substance use relapse (Carmody, Vieten, & Astin, 2007; Cook, McFall, Calhoun, & Beckham, 2007), and smokers with PTSD report greater expectation that smoking will relieve negative affect (Calhoun et al., 2011). Consequently, elevated negative affect in the course of abstinence is particularly worthy of further research.

It is possible that more severe withdrawal in PTSD is due to increased sensitivity to the interoceptive cues that emerge early in the withdrawal process (Feldner et al., 2008). This is consistent with research indicating that smokers with PTSD experience elevated anxiety sensitivity (fear of anxiety-related symptoms), negative affectivity, and anxious arousal (Vujanovic, Marshall-Berenz, Beckham, Bernstein, & Zvolensky, 2010).

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Table 2. Means and *SD*s of Withdrawal Symptoms by PTSD Status and Smoking Condition

	Nicotinized cigarette		Denicotinized cigarette		No smoking		Effect size estimates for overall tests		
	Presmoke	Postsmoke	Presmoke	Postsmoke	Presmoke	Postsmoke	PTSD partial η^2	Smoke partial η^2	PTSD × Smoke partial η^2
	M (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)	*M* (*SD*)			
Craving							.041*	.373*	.012
PTSD	37.2 (6.9)	25.7 (11.3)	36.8 (7.1)	35.9 (7.5)	37.2 (6.0)	37.4 (5.7)			
Non-PTSD	30.1 (8.4)	19.9 (7.2)	29.1 (7.8)	26.4 (7.9)	29.7 (8.6)	29.8 (7.8)			
Negative Affect							.039*	.131*	.056*
PTSD	14.6 (5.6)	12.6 (3.3)	15.3 (5.5)	14.0 (4.6)	15.9 (5.6)	17.5 (4.8)			
Non-PTSD	13.2 (3.4)	12.4 (2.8)	14.4 (4.1)	13.0 (3.4)	14.6 (3.1)	13.9 (4.0)			
Arousal							.067*	.007	.037
PTSD	14.0 (4.5)	13.4 (5.1)	14.1 (3.4)	13.1 (3.7)	13.1 (3.2)	10.9 (3.3)			
Non-PTSD	15.1 (3.6)	14.8 (4.1)	14.1 (4.5)	14.0 (4.0)	14.1 (3.7)	14.7 (4.1)			
Somatic							.006	.011	.012
PTSD	18.0 (5.7)	19.5 (8.2)	17.3 (4.7)	18.5 (5.9)	18.5 (5.1)	19.4 (5.5)			
Non-PTSD	17.0 (5.9)	16.7 (6.7)	16.3 (5.7)	18.2 (9.3)	16.6 (6.0)	16.4 (6.4)			
Appetite							.011	.035	.007
PTSD	8.3 (4.2)	7.7 (3.9)	8.1 (3.4)	7.6 (4.2)	6.4 (3.5)	7.5 (3.9)			
Non-PTSD	5.8 (3.3)	5.1 (2.5)	5.5 (2.7)	5.5 (3.3)	6.5 (3.9)	6.5 (4.1)			
Habit							.071*	.135*	.015
PTSD	11.1 (4.3)	8.6 (4.7)	11.9 (5.0)	10.9 (5.0)	11.3 (4.6)	12.5 (4.2)			
Non-PTSD	8.4 (3.9)	5.8 (2.7)	7.5 (3.2)	6.9 (3.4)	8.5 (3.9)	8.0 (3.7)			

Note. Means were not adjusted for covariates. All significance tests of change in smoking withdrawal model postsmoking withdrawal symptoms as the outcome, with nicotine dependence and presmoking withdrawal symptoms statistically controlled and PTSD, smoking condition, and PTSD × Smoking interaction as the predictors. Significance was tested with Wald significance tests generated by generalized estimating equations. Due to the lack of available effect size statistics for generalized estimating equations, effect sizes were estimated using η^2 calculations from analysis of covariance models.

*$p < .05$.

Each of these could worsen the experience of smoking withdrawal symptoms. Finally, the symptoms that worsened during smoking withdrawal, particularly negative affect and arousal, conceptually overlap with PTSD symptomatology.

This study was limited by the relatively small sample size, necessitating replication. Results are not generalizable beyond the first day of nicotine withdrawal and are also limited by a disparity in sex by PTSD group and overrepresentation of Blacks. Because participants were not trying to quit smoking, results might not generalize to smoking cessation attempts. Finally, although statistical evaluation did not reveal evidence of differential carryover, order effects remain a potential threat to validity in any crossover design. However, this threat is mitigated by the use of large washout periods between smoking condition sessions.

Nonetheless, this study provides preliminary evidence for increased smoking withdrawal among PTSD smokers early in the time course of abstinence. Comprehensive evaluation of withdrawal and smoking urges among PTSD smokers (including affective, attention, and neurocognitive measures) may inform targeted smoking cessation treatment among PTSD smokers. Potential modifications include frequent counseling contact on the quit date, behavioral activation to increase positive affect and reduce negative affect (Hopko, Lejuez, Ruggiero, & Eifert, 2003), and/or precessation nicotine replacement therapy.

Funding

This material is based upon work supported in part by K24DA016388, R21DA019704, R01MH62482, the National Institutes of Health Loan Repayment Program, and the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Clinical Sciences Research and Development and Health Services Research and Development.

Declaration of Interests

None declared.

Acknowledgments

The views expressed in this article are those of the authors and do not necessarily represent the views of the Department of Veterans Affairs, the National Institutes of Health, or the U.S. government.

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Psychopharmacology (2012) 222:701 708
DOI 10.1007/s00213 012 2673 5

ORIGINAL INVESTIGATION

μ-Opioid receptor availability in the amygdala is associated with smoking for negative affect relief

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Received: 27 October 2011 / Accepted: 16 February 2012 / Published online: 3 March 2012
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Abstract
Rationale The perception that smoking relieves negative affect contributes to smoking persistence. Endogenous opioid neurotransmission, and the μ-opioid receptor (MOR) in particular, plays a role in affective regulation and is modulated by nicotine.
Objectives We examined the relationship of MOR binding availability in the amygdala to the motivation to smoke for negative affect relief and to the acute effects of smoking on affective responses.
Methods Twenty-two smokers were scanned on two separate occasions after overnight abstinence using [^{11}C]carfentanil positron emission tomography imaging: after smoking a nicotine-containing cigarette and after smoking a denicotinized cigarette. Self-reports of smoking motives were collected at baseline, and measures of positive and negative affect were collected pre- and post- cigarette smoking.
Results Higher MOR availability in the amygdala was associated with motivation to smoke to relieve negative affect. However, MOR availability was unrelated to changes in affect after smoking either cigarette.
Conclusions Increased MOR availability in amygdala may underlie the motivation to smoke for negative affective relief. These results are consistent with previous data highlighting the role of MOR neurotransmission in smoking behavior.

Keywords Smoking motivation · μ-Opioid receptor · Amygdala · Affect regulation

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Introduction

Tobacco smoking is the leading preventable cause of death worldwide (WHO 2009). Despite this, 20% of adults in the United States continue to smoke (CDC 2009). The perception that smoking relieves negative mood or affect has long been considered a key motive for continued smoking (Kassel et al. 2007; Shiffman 1993). Indeed, negative mood symptoms following a quit attempt predict smoking relapse (Lerman et al. 2002; Strasser et al. 2005b). However, self-report measures of mood and smoking motives have their limitations, and are not always in agreement with real-time

monitoring in smokers (Shiffman et al. 1997; Shiffman and Prange 1988). Assessment of the neurobiological mechanisms that underlie smoking motives related to negative affect regulation could provide insights for treating nicotine dependence (Lerman and Audrain-McGovern 2010).

The endogenous opioid system, and the μ-opioid receptor (MOR) in particular, plays a role in both affective regulation and nicotine dependence (Mague and Blendy 2010; Ribeiro et al. 2005; Walters et al. 2005). There is a high density of MORs in the amygdala and throughout the limbic system (Pfeiffer et al. 1982), and MOR neurotransmission may mediate affective regulation (Ribeiro et al. 2005). Human positron emission tomography (PET) imaging studies have demonstrated positive associations of MOR availability (decreased endogenous opioid neurotransmission) in the amygdala and limbic system with increases in negative affect ratings following negative mood induction (Zubieta et al. 2003), whereas decreases in negative affective ratings after a pain challenge were associated with lower MOR binding availability (Zubieta et al. 2001).

Of relevance to nicotine dependence, preclinical studies have shown that acute nicotine administration modulates release of endogenous opioids (Boyadjieva and Sarkar 1997; Houdi et al. 1991; Marty et al. 1985) and chronic nicotine exposure upregulates MORs (Wewers et al. 1999). In mice, MOR activation is required for nicotine reward (Berrendero et al. 2002; Walters et al. 2005) as well as the anxiolytic effects of nicotine (Balerio et al. 2005). Furthermore, smokers exhibit increased MOR availability in amygdala relative to non-smoking controls (Ray et al. 2011), and a variant in the gene encoding the MOR in humans (*OPRM1* A118G) is associated with the relative reinforcing value of nicotine (Perkins et al. 2008; Ray et al. 2006) as well as with smoking cessation outcomes (Lerman et al. 2004; Munafo et al. 2007; Ray et al. 2007). The MOR antagonist naltrexone mitigates increases in craving and negative affect in abstinent smokers viewing smoking cues (Hutchison et al. 1999), and there is evidence that naltrexone may be an effective smoking cessation treatment among certain subpopulations of smokers (Epperson et al. 2010; O'Malley et al. 2006; Walsh et al. 2008). These data highlight the dual role of the opioid system in affect regulation and nicotine dependence, and suggest a potential neurobiological mechanism that may underlie the motivation to smoke for negative affect relief.

In this secondary analysis of data from a previous [^{11}C] carfentanil PET imaging study (Ray et al. 2011), we examined the relationship of MOR availability to self-reported smoking for negative affect relief. Twenty-two smokers were scanned on two separate occasions (after smoking a nicotine containing cigarette and after smoking a matching denicotinized cigarette). We focused on the amygdala as our primary region of interest and included other limbic system structures (i.e., the thalamus, anterior cingulate cortex, caudate nucleus, insula, and ventral striatum) in a secondary exploratory analysis. Based on prior data described above, we hypothesized that increased MOR binding availability would be associated with increases in self-reported motivation to smoke for negative affect relief. In a secondary analysis, we tested the hypothesis that acute negative affect relief from smoking would be associated with decreased MOR availability (increased endogenous opioid neurotransmission).

Methods and materials

Participants

Participant selection and study design were described previously (Ray et al. 2011). Briefly, smokers were required to report consumption of ≥10 non-menthol cigarettes per day for at least the past 6 months and provide a baseline breath carbon monoxide (CO) reading >10 ppm. The study was approved by the University of Pennsylvania Institutional Review Board, the Food and Drug Administration, and the Environmental Health and Radiation Safety Committee; all participants provided written informed consent. Individuals with a history of or current neurological or DSM-IV Axis I psychiatric or substance disorders (except nicotine dependence), and those taking psychotropic medications were excluded. Of 26 eligible smokers, one smoker was deemed ineligible at PET scan 1 due to noncompliance with the overnight abstinence requirement, one withdrew before the first scan, and two were excluded from the final dataset because a different scanner was used for their sessions, leaving 22 participants with complete data.

Procedures

Following eligibility screening, participants completed measures of smoking history and demographics. The modified version of the Horn–Waingrow Reasons for Smoking (RFS) Scale (Horn and Waingrow 1966) was used to assess smoking motives. Based on our prior work on smoking motives (Lerman et al. 1996; Lerman et al. 1998), we analyzed the three items comprising the RFS subscale "smoking for negative affect reduction" (e.g., "When I feel uncomfortable or upset about something, I light up a cigarette"). This subscale has been shown to correlate with self-monitored smoking behavior (Shiffman and Prange 1988; Tate and Stanton 1990) and has a stable factor structure and satisfactory test–retest reliability (Costa et al. 1980). RFS items were rated on a Likert scale (0="not at all characteristic of me" to 3="very much characteristic of me"); answers to the three items were summed to provide a Negative Affect Reduction summary score (NAR score).

Participants also completed the Fagerstrom Test for Nicotine Dependence (FTND; Heatherton et al. 1991) and the Diener and Emmons Mood Form (Diener and Emmons 1984). This form consists of nine items assessing how strongly subjects are experiencing positive and negative moods; items are rated on a scale of 0 (not at all) to 6 (very much). *OPRM1* A118G (rs1799971) genotype was determined using Taqman SNP genotyping assays (Applied Biosystems) as previously described (Ray et al. 2011).

On each of two separate visits, 60-min [^{11}C]carfentanil PET scans were performed after 14 h of overnight abstinence from smoking (verified by expired CO <10 ppm), as previously described (Ray et al. 2011). Participants completed a urine drug screen at each session to verify abstinence from psychotropic medications. To standardize nicotine exposure, and to avoid nicotine withdrawal, all participants smoked a Quest® research cigarette (Vector Tobacco) 15 min prior to the PET scan, using a standardized puffing procedure (Strasser et al. 2005a). As reported previously (Ray et al. 2011), the scan order was fixed: the nicotine cigarette (0.6 mg nicotine) was smoked before scan 1 and the placebo cigarette (denicotinized; 0.05 mg nicotine) was smoked before scan 2. Participants and data analysts were blind to the cigarette type. Participants also completed the Diener and Emmons Mood Form before and after smoking the cigarette. Female participants were scanned during their early follicular phase (2–9 days after menses) to minimize the effects of rising levels of estrogen on the endogenous opioid system (Mills et al. 2004). The time interval between the two scans was 1–4 weeks (as female participants were scanned during their early follicular phase).

Image acquisition, reconstruction, and preprocessing

Scans were acquired on a GSO-based Phillips GPET Plus brain PET scanner, a custom-built model which operates without septa to increase the sensitivity of the instrument (Karp et al. 2003). Six sequential 10-min-long scans were acquired starting immediately after slow bolus injection (over 10 min) of 3–15 mCi [^{11}C]carfentanil, so as not to exceed 0.03 μg/kg. The dose of [^{11}C]carfentanil administered was based on published PET studies (Greenwald et al. 2007; Smith et al. 2006; Zubieta et al. 2005). The resulting six images consisted of 128 slices of 2-mm slice thickness (2×2 mm^2 pixels) and an image matrix size of 128×128 pixels. The raw PET data were converted to Neuroimaging Informatics Technology Initiative format and preprocessed using SPM8 (Wellcome Trust Center for Neuroimaging, London, United Kingdom) Briefly, the data were first oriented to the AC-PC line and normalized to the MNI T1-weighted template using a 12-parameter affine registration algorithm in SPM8. Nonbrain areas were removed using a brain mask (Ray et al. 2011)

Regions of interest

Left and right hemisphere masks were defined as shown in Fig. 1 for the amygdala and the exploratory regions of interest (ROIs) (thalamus, anterior cingulate cortex, caudate nucleus, insula and ventral striatum) using the Harvard–Oxford probabilistic map distributed with FSL software (Smith et al. 2004). All ROIs except the ventral striatum were defined using a maximal probability threshold of 25%. The ventral striatum/nucleus accumbens ROI was defined using the accumbens label at 0% threshold. Any overlap with the caudate nucleus ROI was removed from the ventral striatum mask. Similarly, ventral striatum was subtracted from the caudate nucleus to create non-overlapping regions of interest.

Quantification of MOR binding (BP_{ND})

MOR availability was calculated with the multilinear reference tissue method MRTM2 (Ichise et al. 2003) with a fixed value for the tissue to plasma efflux rate constant (k_2') in the reference region (occipital cortex). The distribution volume ratio (DVR) is calculated from the ratio of the regression coefficients (Ichise et al. 2003) and the binding potential is given by BP_{ND}=DVR−1. A k_2' value of 0.1237 min ^{1} was assigned on the basis of a previous [^{11}C]carfentanil study (Hirvonen et al. 2009). The MRTM2 BP_{ND} values for each ROI were exported for further statistical analysis using Stata software.

Statistical methods

Descriptive statistics were obtained for all variables. Multiple linear mixed-models (random effects regression) of MOR BP_{ND} were estimated incorporating the NAR score as the predictor and including the following covariates: session, *OPRM1* genotype (A/A=1, */G =0), sex, FTND score, and hemisphere (L=1, R=2). These covariates were chosen to control for potential confounding factors in this analysis: *OPRM1* genotype was previously reported to be significantly associated with MOR BP in this sample (Ray et al. 2011), and other studies have demonstrated associations between MORs and sex (Zubieta et al. 1999) as well as nicotine dependence (Zhang et al. 2006). Next, multiple linear mixed-models (random effects regression) of MOR BP_{ND} were estimated using positive or negative affect difference scores (post- minus pre-cigarette) as the predictor, controlling for the same covariates. The alpha value was set at 0.05 for the bilateral amygdala based on our a priori hypothesis; other ROIs were deemed exploratory and therefore no correction for multiple hypothesis testing was applied.

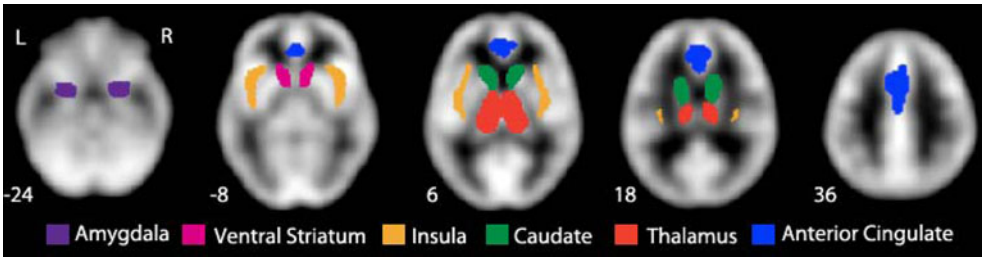


Fig. 1 MOR BP_{ND} masks for each ROI. MOR BP_{ND} was extracted from the amygdala and the five exploratory ROIs for offline analysis

Results

Descriptive data

Of the 22 smokers, six (27%) were female, and all were of European ancestry. Participants reported a mean age of 31.3 years (SD=10.7), mean smoking rate of 17.6 cigarettes per day (SD=6.3), and mean FTND score of 4.68 (SD=2.01). The genotype distribution for *OPRM1* A118G (rs1799971) was 12 A/A, nine A/G, and one G/G; for purposes of analysis, A/G and G/G groups were combined and denoted as */G. The mean NAR score was 4.23 (SD=2.18); Chronbach's alpha estimate for this three-item scale was 0.78.

MOR availability in amygdala and motivation to smoke for negative affect relief

Stronger baseline motivations to smoke for negative affect relief were associated with higher levels of MOR availability in amygdala (β=0.06, 95% confidence interval=0.02–0.10, p=0.007; Fig. 2). As previously reported (Ray et al. 2011), there were no effects of session on MOR BP in the amygdala (p=0.73). The marginal mean MOR BP_{ND} values in the amygdala (adjusted for *OPRM1* genotype, sex, and FTND score) were 1.18 in the nicotine session and 1.17 in the denicotinized session.

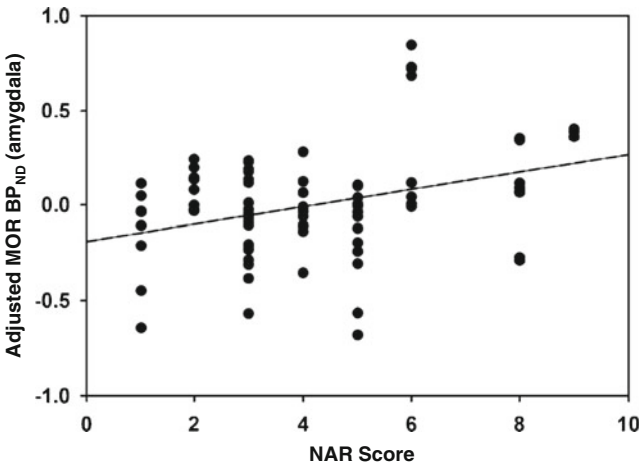


Fig. 2 Association of NAR score with MOR BP_{ND} in the amygdala. Binding potentials for amygdala (adjusted for session, *OPRM1* genotype, sex, FTND, and hemisphere) are plotted for each participant for both sessions. In the multiple regression models, NAR scores were significantly associated with MOR BP_{ND} in the amygdala (β=0.06, p=0.007). *MOR* BP_{ND} μ opioid receptor binding potential, *NAR* Negative Affect Reduction score

In the exploratory analysis, motivation to smoke for negative affect relief was associated with MOR BP_{ND} in the anterior cingulate cortex (p=0.03), but not in the thalamus (p=0.05), caudate nucleus (p=0.27), insula (p=0.59), or ventral striatum (p=0.17).

MOR availability in amygdala and smoking-induced mood change

Smoking the research cigarettes did not produce significant changes in negative or positive mood (as measured using the Diener and Emmons Mood Form) in either session, and across sessions there was no association of positive or negative mood change with MOR availability. Decreased positive mood (post-cigarette minus pre-cigarette) after smoking a nicotine cigarette was associated with increased MOR BP_{ND} in the amygdala, but this effect was not significant (β=−0.14, 95% confidence interval=−0.29–0.01, p=0.08); there was no significant association in the denicotinized cigarette session (p=0.14). Change in negative affect in either session was not significantly associated with MOR BP_{ND} in the amygdala (p>0.10).

Discussion

We report a strong association between MOR availability in the amygdala and motivation to smoke to relieve negative affect. However, contrary to our expectation, there were no significant changes in affect related to smoking the cigarettes prior to the scans, nor was the degree of change in negative affect after smoking related to MOR availability. Thus, we find that a general, pre-existing motivation for smoking relates to MOR binding availability, but that the experience of smoking, at least in this setting, does not.

Our finding of a positive correlation between MOR availability in the amygdala and motivation to smoke for negative affect relief is consistent with prior literature documenting the role of the MOR in both affect regulation and motivational behavior. The association of MOR availability in the amygdala with affective motives to smoke is

consistent with functional neuroimaging evidence for a role of the amygdala in processing and regulating affective responses (Davidson and Irwin 1999; Diekhof et al. 2011; Kim et al. 2011). The amygdala is highly connected to cortical structures throughout the brain and is important in the integration and regulation of emotional responses (Pessoa 2010). Opioid neurotransmission in the amygdala has been implicated in assigning motivational salience to reward cues in rats (Mahler and Berridge 2009), and in humans changes in MOR availability are associated with emotional responses to stimuli (Ribeiro et al. 2005; Zubieta et al. 2003). Other than our previous report on this sample (Ray et al. 2011), only one other study has used PET imaging to examine acute effects of smoking on MOR availability (Scott et al. 2007). Scott and colleagues found a significant increase in MOR availability from the denicotinized to the nicotine condition, and the increase in MOR availability in the amygdala was related to decreased craving scores, but not to mood measures. Although we did administer a two-item craving questionnaire to participants before and after smoking the cigarettes, changes in craving were not related to MOR availability in our sample (data not shown). However, the differing results between these two studies may likely be attributed to differences in study design, including study population (six male smokers in the prior study versus 22 smokers of both genders in the current study), number of cigarettes smoked in each condition (two cigarettes 10 min apart versus a single pre-scan cigarette), and nicotine yield of the nicotine cigarette (1.01 mg versus 0.6 mg).

There are several potential interpretations of finding an association of MOR availability in the amygdala with smoking motivation but not with actual mood change after smoking a cigarette, suggesting future avenues of investigation. It is possible that those individuals with greater MOR availability may have experienced greater negative affect relief early in their smoking experience, and that the belief that cigarette smoking reduced negative affect persisted despite the development of tolerance to pharmacological effects of nicotine. In support of this, adolescents with higher expectancies for negative affect relief from smoking do report greater reductions of negative affect after smoking (Colvin and Mermelstein 2010), whereas expectancies for negative affect relief are not related to actual changes in negative affect in adult smokers (Perkins et al. 2011). Another potential explanation may relate to our use of research cigarettes yielding 0.6 mg of nicotine for the "nicotine cigarette" session, which was lower than the mean yield of our participants' preferred brands (mean=1.1 mg, SD=0.5 mg). Prior studies have shown that low-nicotine cigarettes are rated as lower quality and less satisfying than participants' preferred brands (Benowitz et al. 2006; Strasser et al. 2007). It is possible that the research cigarettes were less rewarding than the participants' usual brand, and thus did not affect mood in the same fashion. In addition, even the denicotinized cigarette contains minute amounts of nicotine; Brody et al. (2009) found that smoking a denicotinized cigarette results in reduced, but still significant, activation of $\alpha 4\beta 2$ nicotinic receptors, which may explain why the smoking manipulation in the current study had no effect. Alternatively, there is evidence to suggest that effects of smoking on mood may derive more from the sensory effects or ritual of smoking than from nicotine (Juliano et al. 2011; Perkins et al. 2008; Rose 2006). Our paced puffing procedure, although necessary to standardize nicotine exposure, did not allow participants to smoke at their own pace or to smoke to satiety, and thus may have affected mood differently than self-regulated smoking. Future investigations into smoking-related mood effects may benefit from assessments of mood change in a naturalistic environment using the participants' preferred cigarettes.

Another possibility to consider is that it is μ-opioid system regulation of reward, rather than mood change, which drives the motivation to smoke to relieve negative affect. There is a growing body of literature suggesting that although smokers expect improvements in mood after smoking a cigarette, smoking does not reliably relieve negative affect due to anything other than nicotine withdrawal (Conklin and Perkins 2005; Perkins et al. 2010). Indeed, the absence of significant mood change in our study is consistent with this literature. However, a common polymorphism in the gene encoding the MOR (*OPRM1* A118G) is associated with the relative reinforcing value of nicotine (Perkins et al. 2008; Ray et al. 2006). Interestingly, the A allele, which we previously demonstrated was associated with greater MOR availability in this sample (Ray et al. 2011), has also been associated with increased cigarette reward during negative versus positive mood (Perkins et al. 2008) as well as with increased reinforcement learning compared to G allele carriers (Lee et al. 2011). It is possible that even though smoking is not effective in actually relieving negative mood, smokers with greater MOR availability find cigarettes more rewarding under these circumstances and are more likely to develop a conditioned association between smoking and negative mood.

There are a few limitations which should be considered within our study. The NAR scale is a self-report measure and relies on accurate self-assessment of motivation rather than objective observation; however, this measure has been shown to correlate significantly with self-monitored smoking data (Shiffman and Prange 1988; Tate and Stanton 1990). Mean NAR scores in this study were lower than scores for non-depressed smokers in previous studies (Lerman et al. 1996; Lerman et al. 1998). This may be related to oversampling for *OPRM1* */G genotype, which has been associated with reduced subjective nicotine reward (Ray et al. 2011) and reduced reinforcement learning (Lee

et al. 2011). Also due to oversampling of *OPRM1* */G, the distribution of *OPRM1* genotypes in our sample is not representative of the normal smoking population. Smokers of menthol cigarettes were excluded from the study; therefore caution should be used when extrapolating to general smoking populations. We did not use a smoking topography device to measure puff volume or assess plasma nicotine levels at either session. Our use of a standardized puffing procedure mitigates differences in puff volume between smokers (Strasser et al. 2005a), and significant differences in plasma nicotine levels are obtained when smoking Quest® denicotinized cigarettes compared to the 0.6 mg cigarettes (Brody et al. 2009). Other studies have shown variation in MOR availability and in μ-opioid system response to stimuli between males and females (Zubieta et al. 1999; Zubieta et al. 2002). The number of female smokers included in the current study was too small to test for sex heterogeneity; however, sex was included as a covariate in all models and did not predict MOR availability. Finally, as with any PET imaging study, the reported binding potentials do not distinguish between number of available receptors, receptor affinity or endogenous opioid tone.

In conclusion, we have demonstrated a significant association of MOR availability in the amygdala with motivation to smoke to relieve negative affect; furthermore, this motivation was inconsistent with the actual experience of smoking. These data offer a potential biological mechanism underlying this particular smoking motive, and add to a growing body of literature highlighting inconsistencies between smokers' expectations and real-time effects of smoking. Further research into the biological mechanisms influencing affective response to smoking and motivation to smoke may be useful in effecting improvements in smoking cessation treatment.

Acknowledgements We thank the following individuals for their contributions to the study: Dr. Richard Freifelder, Dr. Joel Karp, Dr. Alexander Schmitz, and Rahul Poria for [^{11}C]carfentanil synthesis; Dr. Daniel Pryma and Dr. Rodolfo Perini for serving as PET center injectors; and Dr. Janet Reddin and PET center technologists for PET acquisition and preprocessing at the PET center. This research was supported by National Institute on Drug Abuse Grants R21 DA027066 (to C.L. and J.A.B.) and U01 DA020830 (to C.L.), National Cancer Institute Grant P50 CA143187 (to C.L. and J.A.B.), and a grant from the Pennsylvania Department of Health. C.L. has served as a consultant for and/or received research support from Pfizer, AstraZeneca, Novartis, and GlaxoSmithKline. R.R. has received research support from Pfizer. This research was not supported by industry funds. The authors declare that they have full control of all primary data and they agree to allow the journal to review their data if requested.

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Experimental and Clinical Psychopharmacology
2012, Vol 20, No 4, 278–286

© 2012 American Psychological Association
1064-1297/12/$12 00 DOI: 10 1037/a0028652

A Direct Test of the Influence of Nicotine Response Expectancies on the Subjective and Cognitive Effects of Smoking

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Regardless of actual nicotine content, expectations about the nicotine content of a cigarette influence the rewarding subjective effects of smoking, and may even affect cognitive performance. These effects are theorized to be mediated by beliefs about effects of cigarette smoking, or response expectancies. However, few studies have directly manipulated response expectancies. Understanding the effects of such manipulations could improve effectiveness of nicotine-dependence treatments and medications. Using a 2 × 2 between-subjects factorial design, cigarette smokers ($N = 80$) smoked either a nicotine or a placebo (denicotinized) cigarette crossed with instructions that the cigarette would either enhance or impair cognitive and motor performance. As predicted, participants in the "told enhance" condition reported significantly greater beliefs that nicotine had beneficial effects on performance than those in the "told impair" condition. Compared to those "told impair," those "told enhance" reported more psychological reward, enjoyable physical sensations, and craving reduction from the cigarette, as well as greater motivation to perform well on a cognitive task. Relative to placebo cigarettes, nicotine cigarettes produced greater reports of satisfaction, craving reduction, and dizziness. Smoking a nicotine cigarette produced better performance on the Rapid Visual Information Processing Task, a test of sustained attention; but the expectancy manipulation had no effect. These data suggest that response expectancies can be experimentally manipulated and can influence perceived rewarding effects of cigarette smoking, but do not appear to affect cognitive performance. These findings add to our understanding of the benefits and limitations of expectancy manipulations, both experimentally and as a treatment technique.

Keywords expectancies, nicotine, cognitive, Rapid Visual Information Processing Task, placebo effects

It has been suggested that placebo or nocebo effects result from expectations individuals have about a drug or treatment. The knowledge that one has been administered a drug or treatment is referred to as a *stimulus expectancy*, whereas idiosyncratic beliefs about the effects that the drug or treatment will have on one's feelings, cognitions, or behaviors are referred to as *response expectancies* (Kirsch, 1985; Perkins, Sayette, Conklin, & Cagguila, 2003). According to expectancy-based conceptualizations of placebo effects, the belief that one has consumed a drug activates response expectancies, which directly produce responses or symptoms consistent with the expected effects of the drug (Kirsch, 1999). It is believed that response expectancies not only affect reactions to placebos, but also influence the pharmacological effects of drugs (Kirsch, 1999; Perkins et al., 2003). Investigating the mechanisms of placebo effects provides an opportunity to learn more about mind−body interactions and may uncover ways to control placebo effects to improve the effectiveness of treatments and medications (Copeland & Brandon, 2000; Finniss, Kaptchuck, Miller, & Bennedetti, 2010; Fucito & Juliano, 2007).

There is growing evidence that the rewarding and reinforcing effects of cigarette smoking may be due in part to placebo effects. Studies using the balanced placebo design, which crosses actual drug exposure (nicotine vs. placebo cigarettes) with expected drug exposure (told nicotine vs. placebo), have demonstrated that expecting nicotine influences responding to placebo (denicotinized) and nicotine cigarettes. In general, expecting nicotine in a cigarette, regardless of actual drug content, produces increased smoking behavior (Perkins et al., 2008), reduced urges and cravings to smoke (Kelemen & Kaighobadi, 2007; Perkins et al., 2008), improved mood (Juliano, Fucito, & Harrell, 2011), greater smoking satisfaction (Juliano et al., 2011; Kelemen & Kaighobadi, 2007; Perkins et al., 2004; Perkins et al., 2008), and a variety of other self-reported positive responses (e.g., greater wakefulness, concentration) compared to expecting placebo. Furthermore, a recent study demonstrated that expecting nicotine resulted in fewer errors on the Rapid Visual Information Processing (RVIP) task, a computerized cognitive task assessing sustained attention (Juliano et al., 2011). Thus, there is mounting evidence that subjective, affec-

This article was published Online First June 18, 2012.

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This research was supported by Mellon funds provided by the College of Arts and Sciences at American University and NIH grant T32-DA007292. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Drug Abuse or the National Institutes of Health. We would like to thank Zandra Zweber, Lisa Fucito, and Peter Kardel for their assistance with data collection and Andrew J. Waters, Clara M. Cheng, and Scott Parker for helpful feedback on earlier drafts of this manuscript.

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tive, behavioral, and perhaps even cognitive effects of smoking may be in part due to expectancy effects. At present, it is unclear how stimulus expectancies exert these effects. It may be related to response expectancies. However, smoking research, like most research investigating drug-related placebo responding, has focused primarily on manipulating stimulus expectancies, with less emphasis on manipulating or evaluating response expectancies. *Self-report* measures of smoking response expectancies have been included in some placebo research. For example, Juliano and Brandon (2002) found that expecting nicotine during smoking produced greater anxiety reduction than expecting placebo, but only among smokers who reported greater expectancies that smoking alleviates negative affect. Another investigation found that self-reported response expectancies for the performance-enhancing effects of cigarette smoking assessed after smoking and completing a cognitive performance task (i.e., RVIP) were associated with actual performance in some cases (Kelemen, 2008). Studies involving caffeine have shown that participants' beliefs about the effects of caffeine can predict subjective or performance-enhancing effects of decaffeinated coffee (e.g., Fillmore & Vogel-Sprott, 1992; Flaten, Aasli, & Blumenthal, 2003; Kirsch & Wiexel, 1988). However, because response expectancies were not manipulated in any of these studies, their causal role in placebo responding cannot be determined.

There is a fairly limited body of research involving direct manipulations of the expected effects of a drug to determine the resulting effects on drug and placebo responding. Two prior studies involving nicotine replacement therapy (NRT) found that manipulations designed to alter expectancies for nicotine replacement resulted in differential reactions to placebo NRT (Fucito & Juliano, 2007; Tate, Stanton, Green, & Schmitz, 1994). Participants given information about NRT benefits reported greater overall vigor when on placebo NRT (Fucito & Juliano, 2007). Similarly, participants who drank coffee and were told that caffeine enhances cognitive performance reported greater performance motivation and fewer negative somatic effects than those told caffeine impairs performance (Harrell & Juliano, 2009). Furthermore, manipulating the expected effects of caffeine or alcohol (i.e., participants told that the drug would either enhance or impair performance) has been shown to influence cognitive and motor performance, resulting in effects consistent with the manipulation for adults given decaffeinated coffee (Fillmore, Mulvihill, & Vogel-Sprott, 1994; Fillmore & Vogel-Sprott, 1994) and in the opposite direction of the manipulation for participants in caffeine withdrawal (Harrell & Juliano, 2009) or receiving alcohol (Fillmore et al., 1994; Fillmore, Roach, & Rice, 2002). The present study was designed to evaluate the influence that expectancies for the effects of cigarette smoking have on reactions to smoking. We were particularly interested in expectancies for the cognitive-enhancing effects of cigarette smoking. Various aspects of cognitive performance, including attention and memory, are enhanced after the administration of nicotine compared to placebo, especially after a period of nicotine deprivation (Heishman, Kleykamp, & Singleton, 2010). Furthermore, smoking for cognitive enhancement is rated as one of the most common motives for smoking (Gilbert, Sharpe, Ramanaiah, Detwiler, & Anderson, 2000). Smoking levels increase during times of high cognitive load, such as before an exam (Patterson, Lerman, Kaufmann, Neuner, & Audrain-McGovern, 2004), and the cognitive and affective effects of nicotine are likely connected in systematic ways (Waters & Sutton, 2000), so that smoking for affective regulation may be related to beliefs about cognitive enhancement, for example, decreasing feelings of anxiety by enhancing perceived coping ability (Kassel & Shiffman, 1997). Given that cigarette smokers often smoke as a means of enhancing or maintaining consistent levels of performance, challenging performance response expectancies may be an effective treatment tool.

The goal of the present study was to determine whether response expectancies for cigarette smoking could be manipulated and if such expectancies directly influenced the rewarding effects of cigarette smoking. In this 2 × 2 factorial study, we manipulated smokers' expectancies for the performance effects of nicotine by informing some participants that smoking enhances cognitive performance and others that it impairs cognitive performance. We also controlled actual nicotine exposure with some participants smoking cigarettes with nicotine and others smoking placebo (denicotinized) cigarettes. As such, participants were randomly assigned to one of the following four conditions: (a) told enhance/given nicotine; (b) told enhance/given placebo; (c) told impair/given nicotine; and (d) told impair/given placebo. Performance expectancies, cognitive performance, cigarette ratings, smoking urge, performance motivation, and resistance to drug effects were assessed. The RVIP task, a commonly used test of sustained attention, was used to assess cognitive performance because this task has been shown to be sensitive to the effects of nicotine (Heishman et al., 2010; Prichard & Robinson, 1998) and, in a prior study, was sensitive to the effects of a stimulus expectancy manipulation (Juliano et al., 2011). Smokers who were told that smoking enhances performance were expected to report more positive nicotine performance expectancies, demonstrate greater cognitive performance, and report more positive reactions to the cigarette (e.g., satisfaction), lower urges to smoke, more motivation to perform well, and less resistance to drug effects, relative to those *told impair*. Smokers *given nicotine* were expected to perform better on the test of sustained attention than those *given placebo*. We were also interested in potential interactions between the expectancy manipulation and cigarette type, including the possibility of a replication of prior findings that participants *told impair* performed better than those *told enhance*, but only for those *given placebo* (Harrell & Juliano, 2009).

Method

Participants

Participants were recruited from the university community (37%) and the Washington, DC, metropolitan area (63%). Participants were required to be at least 18 years old and to smoke between 6 and 40 cigarettes per day. Individuals with chronic smoking-related diseases (e.g., emphysema) were excluded from participation. Participants (N = 80, 30.0% women, M_{age} = 32.8 years, SD = 13.5) smoked a mean of 14.2 (SD = 7.0) cigarettes per day. The mean Fagerström Test of Nicotine Dependence (FTND) score was 4.1 (SD = 2.3). Most of the participants identified themselves as either African American or Black (44%) or White (43%). Table 1 shows demographic and other characteristics of the sample.

Table 1
Premanipulation Values by Condition

	Conditions (told/given)			
	Enhance/nicotine	Enhance/placebo	Impair/nicotine	Impair/placebo
Values	*n* (%) or *M* (*SEM*)	*n* (%) or *M* (*SEM*)	*n* (%) or *M* (*SEM*)	*n* (%) or *M* (*SEM*)
Female	3 (15.8%)	8 (40.0%)	7 (38.9%)	6 (26.1%)
Age (years)*	34.21 (2.96)	26.17 (3.04)	39.30 (2.88)	31.30 (2.69)
Cigarettes per day	14.37 (1.62)	13.06 (1.67)	15.73 (1.58)	13.50 (1.48)
Expectancy	4.87 (0.22)	5.40 (0.22)	5.04 (0.21)	5.23 (0.20)
Withdrawal (MNWS)	1.06 (0.14)	0.80 (0.14)	0.69 (0.14)	0.78 (0.13)
FTND	4.16 (0.52)	3.39 (0.54)	4.35 (0.51)	4.22 (0.47)
Urge	5.40 (0.38)	5.41 (0.39)	5.40 (0.37)	5.51 (0.35)

Note. MNWS = Minnesota Nicotine Withdrawal Scale; FTND = Fagerström Test of Nicotine Dependence.
* Those given nicotine cigarettes were significantly older ($M = 36.82$ years, $SD = 14.21$) than those given placebo cigarettes ($M = 29.05$ years, $SD = 11.70$), $F(1, 79) = 7.68$, $p = .007$, $\eta^2 = 0.09$.

Materials

Experimental cigarettes. The experimental cigarettes were marketed under the trade name Quest (Vector Tobacco, Timberlake, NC). The nicotine cigarette contained a yield of 0.6 mg of nicotine and 10 mg of tar (Quest 1) and the placebo cigarette contained a yield of no more than 0.05 mg nicotine and 10 mg of tar (Quest 3). Nicotine yield refers to measurements of nicotine yield in mainstream smoke by the U.S. Federal Trade Commission method (Hatsukami et al., 2010). It is important to note that nicotine delivery was not directly measured (Donny, Houtsmuller, & Stitzer, 2007). The actual tobacco rods of Quest 1 cigarettes contain a total of 8.9 mg of nicotine, whereas Quest 3 cigarettes contain 0.48 mg of nicotine (Becker, Rose, & Albino, 2008). The dose absorbed from Quest 3 or similar denicotinized cigarettes produces pharmacological effects that are arguably insignificant (Pickworth, Fant, Nelson, Rohrer, & Henningfield, 1999; but see Barrett, 2010; Brody et al., 2009; Gross, Lee, & Stitzer, 1997). Participants were given either a menthol cigarette (47%) or nonmenthol cigarette (53%), depending on their usual smoking preferences. All cigarettes were blinded and coded by an experimenter who had no contact with participants. Prior research has shown than participants can be convinced they are smoking nicotine cigarettes when denicotinized cigarettes are administered (Juliano et al., 2011).

Carbon monoxide. Carbon monoxide (CO) levels were measured in parts per million (ppm) using a Micro III Smokerlyzer (Bedfront Scientific, Kent, England). Breath CO samples were taken to encourage compliance with instructions to abstain from smoking for 12 hr prior to arrival. Presmoking to postsmoking changes in CO level also provided a rough measure of the amount of smoke inhaled during the experimental manipulation.

Performance Measures

RVIP task. The RVIP is a widely used test of sustained attention (or vigilance) that has been shown to be sensitive to the effects of nicotine (Heishman et al., 2010; Juliano et al., 2011). Participants viewed a series of single digits presented on the computer screen at a rate of 100 digits per min for 12 min. Participants were told to press a mouse button as quickly as possible whenever they detected three consecutive odd or three consecutive even numbers. To increase motivation for the RVIP task on the experimental day and to improve sensitivity, participants were informed that they would earn 3 cents for each correct detection (hit) and lose 3 cents for each false alarm. Response targets appeared eight times per min with 8−36 digits appearing between each target. Reaction time and responses were recorded by the computer. Responses that occurred 100−1,500 ms after the target were scored as hits. Sensitivity, which takes into account the number of both hits and false alarms was computed as $A' = 0.5 + [(hr - far) + (hr - far)^2]/4*hr*(1 - far)]$ (Sahgal, 1987), where hr is hit rate and far is false alarm rate. The RVIP task was administered using DirectRT software (Empirisoft, New York).

Finger tapping. Tapping, a measure of psychomotor speed described previously (Harrell & Juliano, 2009), was originally included. However, software problems led to large amounts of missing data and thus the data were not interpretable.

Self-Report Measures

Demographics and smoking history questionnaire. A 15-item questionnaire was developed for this study to assess demographic information (e.g., sex, age, race) and smoking history. It also included the FTND (Heatherton, Kozlowski, Frecker, & Fagerström, 1991), a widely used and validated 6-item measure of nicotine dependence.

Minnesota Nicotine Withdrawal Scale. The Minnesota Nicotine Withdrawal Scale (MNWS), a standardized 8-item measurement of nicotine withdrawal symptomatology as defined by the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (Hughes & Hatsukami, 1986; Toll, O'Malley, McKee, Salovey, & Krishnan-Sarin, 2007), was modified to expand contracted items (i.e., one item: "irritability, frustration, or anger" was expanded to three separate items), yielding 13 items (average Cronbach's alpha = .83). Items were rated on a 5-point rating scale from 0 (*none*) to 4 (*severe*). The mean of all 13 items is used to measure withdrawal.

Cigarette Evaluation Scale. The Cigarette Evaluation Scale (Rose, Behm, & Westman, 2001) assessed participants' immediate reactions to smoking. A total of 10 items were rated using a 7-point scale, ranging from 1 (*not at all*) to 7 (*extremely*). Based on Rose

et al. (2001), two items were combined as a measure of satisfaction ("Was it satisfying?"; "Was there a good taste?"; Cronbach's alpha = .78) and four items were combined as a measure of psychological reward ("Did it calm you down?", "Did it make you feel more awake?", "Did it reduce your hunger for food?", "Did it make you feel less irritable?"; Cronbach's alpha = .78). A third factor noted in Rose et al. (2001), consisting of the items nausea and dizziness, had low internal consistency (0.41), so these two items were analyzed separately. In addition to the eight items above, the questionnaire included two additional items measuring craving relief and enjoyment of airway sensations.

Expectancy measurement. Based on prior similar research on caffeine and alcohol (Fillmore & Vogel-Sprott, 1994; Harrell & Juliano, 2009), participants were asked about their expectancies for the general effects of nicotine on hand−eye coordination as well as their expectancies for specific aspects of task performance (e.g., improve accuracy on the RVIP task). For general expectancies, participants were asked to rate the statement, "I believe nicotine has the following effect on hand−eye coordination and speed." For task-specific expectancies, participants were asked to rate a number of expectancy statements about specific aspects of the tasks (RVIP and tapping). For example, "I expect that nicotine [would/did] have the following effect on my [measure] performance on the RVIP (or tapping) task." Response options ranged from 1 (*largely impair*) to 9 (*largely enhance*). These items were administered both before and after the expectancy manipulation. Measures included "speed" (RVIP and tapping), "accuracy" (RVIP), and "avoid slowing down" (tapping). The mean rating on all five items was used as the measure of expectancy for nicotine effects on task performance. Cronbach's alpha averaged .77.

Urge rating. Three items (Kozlowski, Pillitteri, Sweeney, Whitfield, & Graham, 1996) from the Questionnaire of Smoking Urges (Tiffany & Drobes, 1991) were used to assess participants' cravings, wants, and desires to smoke on a 7-point Likert scale, ranging from 1 (*not at all*) to 7 (*extremely*). The Cronbach's alpha in this study averaged .93.

Task motivation and drug resistance. Immediately after finishing the RVIP task for the final time, participants were asked to compare how motivated they were after the manipulation with their motivation level before the manipulation. They rated their motivation on a 5-point scale, ranging from 1 (*much less than earlier today*) to 5 (*much more than earlier today*). Previous research on caffeine, using a similar manipulation, found an increase in participants' task motivation after "told enhance" instructions relative to "told impair" instructions (Harrell & Juliano, 2009). Drug resistance was also assessed based on prior expectancy research (Fillmore et al., 1994; Harrell & Juliano, 2009; also see Fillmore et al., 2002). Participants were asked, "How much did you try to resist any effects of the nicotine?" and answered on a scale ranging from 0 (*not at all*) to 10 (*extremely*).

Procedure

Interested volunteers were screened by telephone. Eligible participants attended a baseline visit prior to the experimental day to collect baseline information and practice the RVIP to ensure sufficient task comprehension and to reduce the influence of practice effects during the experimental session. Participants were told to smoke as they normally would before their first visit. On arrival, participants gave informed consent, provided a CO sample, and completed self-report and performance measures. To maximize power, a CO cutoff was not used. Participants also smoked one of their own cigarettes ad libitum and rated the cigarette.

The experimental session was scheduled within the next 2 days. Because research has shown that cognitive effects of nicotine are more robust after periods of nicotine withdrawal (Heishman et al., 2010), participants were instructed to abstain from nicotine for 12 hr prior to the second visit. On arrival, participants provided a CO sample and were asked to report the time of their last cigarette. Next they completed premanipulation self-report and performance measures. The participant was then given either a nicotine or a placebo (denicotinized) cigarette to smoke ad libitum, crossed with one of two types of instructions designed to manipulate the expected effects of smoking (performance enhancement vs. performance impairment), resulting in four conditions: (a) *told enhance/given nicotine*; (b) *told enhance/given placebo*; (c) *told impair/given nicotine*; and (d) *told impair/given placebo.*

Instructions appeared on the computer screen and were also read aloud to the participants by the experimenter. Instructions, based on prior research with caffeine (Fillmore & Vogel-Sprott, 1992; Harrell & Juliano, 2009), were as follows:

> This cigarette contains a fairly strong dose of nicotine so that we can observe sizable effects in the shortest period of time. As mentioned earlier, the purpose of this experiment is to assess the effects of nicotine on motor performance.
>
> **Told enhance group.** A number of recent studies have reported that cigarettes improve performance in tasks that involve quick responses to visual stimuli, like video games or data entry. Our tasks involve all of the same skills. Because the effects obtained before were with small doses, we expected to see much stronger enhancing effects of nicotine in the laboratory with this large dose of nicotine. Administering large amounts of nicotine to produce enhancement of performance allows us to understand how nicotine causes this improvement.
>
> **Told impair group.** Recent research has found that nicotine has a negative effect on hand−eye coordination and speed. A number of studies have reported that cigarettes impair performance in tasks that involve quick responses to visual stimuli, like video games or data entry. Our tasks involve all of the same skills. Many people report these impairing effects. They seem to still learn the task, but learning is less efficient. Because the effects obtained before were with small doses, we expected to see much stronger disruptive effects of nicotine in the laboratory with this large dose of nicotine. Administering large amounts of nicotine to produce impairment of performance allows us to understand how nicotine causes this disruption.

After smoking, participants again completed self-report and performance measures. Then they rated how motivated they were to perform the task, relative to both earlier that day and on the prior visit. Finally, participants were asked to rate their experience in the study using a standard experimental evaluation form. Another CO sample was obtained and participants were compensated $30 plus additional compensation based on their performance on the RVIP tasks (up to $5.76). This was the first time any feedback about task performance was provided. Participants were then debriefed about the purpose of the study.

Results

Baseline Data

A series of 2 × 2 (Told Enhance vs. Told Impair × Given Nicotine vs. Given Placebo) analyses of variance and chi squares, when relevant, were conducted to check for baseline equivalence. Alpha was set at $p < .05$. There were no significant differences on any baseline or premanipulation measures with the exception of age. Baseline values are presented in Table 1. Despite random assignment, those given nicotine cigarettes were significantly older ($M = 36.82$ years, $SD = 14.21$) than those given placebo cigarettes ($M = 29.05$ years, $SD = 11.70$), $F(1, 79) = 7.68$, $p = .007$, $\eta^2 = .09$. To control for this baseline difference, age was entered as a covariate in all statistical analyses. It is notable that there were no baseline differences in expectancies for the performance-enhancing effects of nicotine ($p = .986$). In fact, prior to the manipulation, the mean expectancy ratings were nearly identical in the told enhance ($M = 5.14$) and told impair ($M = 5.13$) conditions. Sex was initially included in the analyses, but no significant effects of sex were found. Thus, the results reported do not include sex.

Main Outcomes

RVIP performance. A series of 2 × 2 (Enhance vs. Impair × Nicotine vs. Placebo) analyses of covariance (ANCOVAs) were conducted on RVIP task indices, controlling for premanipulation performance and age. Contrary to predictions, there were no effects of the expectancy manipulation on RVIP performance. However, as shown in Figure 1a, participants who smoked a nicotine cigarette had a significantly greater number of hits on the RVIP, $F(1, 78) = 4.63$, $p = .04$, $\eta^2 = .032$, and showed greater sensitivity, $F(1, 78) = 6.14$, $p = .02$, $\eta^2 = .027$, as compared to those who smoked a placebo cigarette. There were no effects of nicotine on RVIP reaction time.

Cigarette ratings. A series of 2 × 2 (Enhance vs. Impair × Nicotine vs. Placebo) ANCOVAs were conducted on cigarette ratings, controlling for baseline ratings of the participant's own cigarette at the initial visit as well as age. Findings are summarized in Table 2. Compared to the told impair condition, those told enhance reported greater psychological reward ($p = .047$), enjoyable sensations in throat and chest ($p = .045$), and less craving ($p = .020$). Relative to placebo cigarettes, those who smoked nicotine cigarettes reported greater satisfaction ($p = .002$), dizziness ($p = .010$), and less craving ($p = .039$). There were no interactions between drug and expectancy factors.

Urge rating. A 2 × 2 (Enhance vs. Impair × Nicotine vs. Placebo) ANCOVA, controlling for baseline urge and age, found no significant effects for nicotine or expectancy on urge ratings, or any interactions.

Motivation and drug resistance. Based on prior published research findings (Fillmore et al., 1994; Harrell & Juliano, 2009), a series of 2 × 2 (Enhance vs. Impair × Nicotine vs. Placebo) ANCOVAs, adjusted for baseline differences in age, were conducted on ratings of motivation and resistance. As shown in Figure 1b, there was a main effect of expectancy with those told enhance reporting significantly more motivation for performance on the postmanipulation RVIP ($M = 3.50$, $SE = 0.18$) than those told impair ($M = 2.90$, $SE = 0.17$), $F(1, 79) = 6.10$, $p = .02$, $\eta^2 = .07$. There was neither an effect of nicotine nor a significant expectancy by nicotine interaction effect on reported motivation. There were no effects of the manipulations on ratings of drug resistance.

Manipulation Checks

Expectancy manipulation. A 2 × 2 (Enhance vs. Impair × Nicotine vs. Placebo) ANCOVA was conducted on postmanipulation expectancies for the performance-enhancing effects of nicotine, controlling for age premanipulation expectancies for the performance-enhancing effects of nicotine and baseline differ-

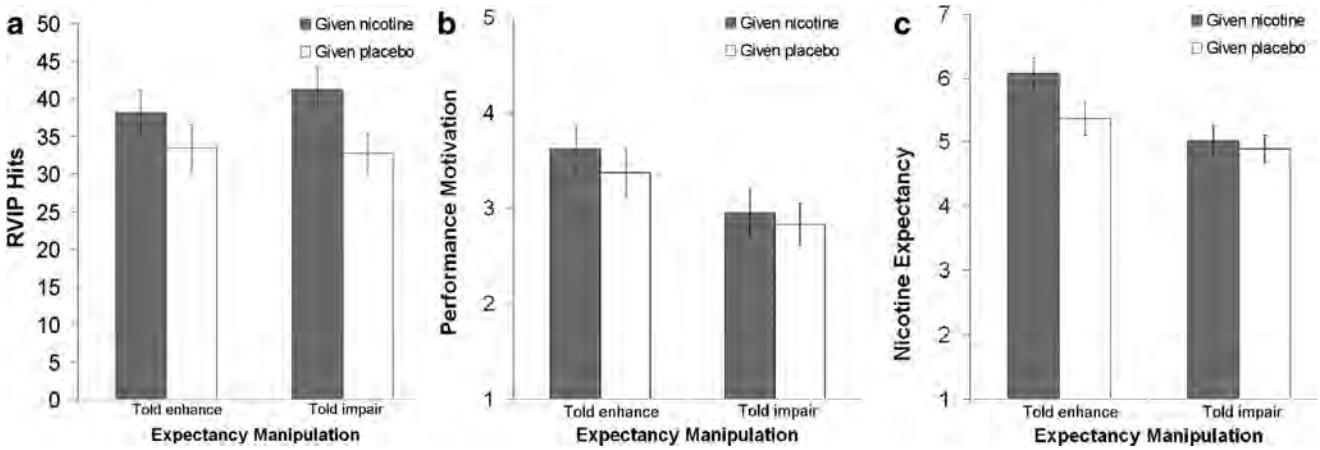


Figure 1. Postmanipulation Rapid Visual Information Processing (RVIP) task hits by condition adjusted for age and premanipulation RVIP hits (a). Postmanipulation RVIP performance motivation by condition adjusted for age. Motivation rated on a 5-point scale, ranging from 1 (*much less than earlier today*) to 5 (*much more than earlier today*) (b). Postmanipulation nicotine performance response expectancy by condition adjusted for age and premanipulation nicotine performance response expectancy (c).

Table 2
Covariate Adjusted Cigarette Ratings Across the Experimental Conditions

	Conditions (told/given)				Effects	
	Enhance/nicotine	Enhance/placebo	Impair/nicotine	Impair/placebo	Main effect nicotine	Main effect expectancy
Conditions	M^a (*SEM*)	M^a (*SEM*)	M^a (*SEM*)	M^a (*SEM*)	*F*, η^2	*F*, η^2
Satisfaction	3.82 (0.29)	3.12 (0.31)	3.89 (0.30)	2.66 (0.27)	$F = 10.15,^{***}$ 0.102	*ns*
Psychological reward	3.00 (0.21)	2.82 (0.23)	2.54 (0.22)	2.41 (0.20)	*ns*	$F = 4.07,^{*}$ 0.032
Dizziness	3.25 (0.32)	2.13 (0.34)	2.80 (0.33)	2.16 (0.29)	$F = 7.00,^{**}$ 0.075	*ns*
Nauseous	1.53 (0.22)	1.22 (0.24)	1.49 (0.23)	1.51 (0.20)	*ns*	*ns*
Reduced craving	4.79 (0.40)	4.21 (0.42)	4.12 (0.41)	2.95 (0.37)	$F = 4.43,^{*}$ 0.039	$F = 5.67,^{*}$ 0.050
Enjoyable sensations in throat and chest	3.41 (0.30)	3.28 (0.32)	3.12 (0.31)	2.34 (0.27)	*ns*	$F = 4.17,^{*}$ 0.033

Note. There were no significant interaction effects.
[a] Adjusted for baseline ratings and group differences in age.
$^{*}p < .05$. $^{**}p < .01$. $^{***}p < .001$.

ences in age. As shown in Figure 1c, participants in the told enhance condition reported significantly greater postmanipulation expectancies for the performance-enhancing effects of nicotine ($M = 5.72$, $SE = .17$) than those in the told impair condition ($M = 4.96$, $SE = .16$), $F(1, 79) = 10.26$, $p = .002$, $\eta^2 = .09$. In terms of absolute change, paired-sample *t* tests showed that participants in the told enhance condition showed a significant increase in expectancies for the performance-enhancing effects of nicotine (+0.65), $t(36) = 3.18$, $p = .003$, and participants in the told impair condition showed a decrease (−0.23), $t(42) = -1.47$, $p = .148$, that was not significant.

Withdrawal. To determine the effects of cigarette abstinence on the participants, paired *t* tests were conducted comparing reports on the practice session to reports at the beginning of the experimental session, prior to cigarette smoking. Participants reported significantly more hours of abstinence on the experimental session, with instructions to abstain for 12 hr ($M = 13.57$, $SE = 0.29$) than on the practice session, under ad lib smoking instructions ($M = 2.54$, $SE = 0.91$), $t(79) = 12.18$, $p < .001$. CO readings were significantly lower in the experimental session ($M = 9.18$, $SE = 0.96$) than in the practice session ($M = 17.10$, $SE = 1.25$), $t(79) = -8.04$, $p < .001$. Urge ratings were significantly higher in the experimental session ($M = 5.43$, $SE = 0.18$) compared to the practice session ($M = 4.30$, $SE = 0.23$), $t(79) = 4.77$, $p < .001$. No effects were found for the MNWS. Because the lack of a finding for the MNWS was unexpected, a paired *t* test comparing the MNWS pre- and postcigarette was conducted. The results indicated a significant drop postcigarette, $t(75) = 7.83$, $p < .001$. This effect was found for both the given nicotine group, $t(38) = 5.73$, $p < .001$, and the given placebo group, $t(36) = 5.47$, $p < .001$.

Smoking behavior. A 2 × 2 × 2 mixed-factor ANCOVA was performed with breath CO (pre- and postsmoking) as the within-subjects factor, dose expectancy (enhance vs. impair) and nicotine dose (nicotine vs. placebo) as between-groups factors, and age included as a covariate. As expected, CO levels increased significantly from an average of 9.09 ppm ($SE = 1.04$) prior to smoking the cigarette to 13.24 ppm ($SE = 0.98$), approximately 30 min after smoking the cigarette, $F(1, 69) = 19.09$, $p < .001$. There were no other main effects or interactions indicating differences in smoking exposure based on the experimental manipulations.

Discussion

This study used a 2 × 2 between-subjects factorial design to evaluate changes in the subjective and performance-enhancing effects of cigarette smoking resulting from manipulations of nicotine response expectancies and actual nicotine exposure. As hypothesized, daily cigarette smokers reported changes in their expectancies for nicotine's effects on performance consistent with response expectancy manipulations. Smokers told that nicotine enhances performance reported that the experimental cigarette, irrespective of its nicotine content, was more psychologically rewarding, more effective at reducing cravings, and caused more enjoyable physical sensations. Furthermore, participants expecting enhancement reported greater motivation than participants told that performance would be impaired, but there was no difference between the two groups in reported efforts to resist drug effects. Contrary to predictions, there was no effect of the expectancy manipulation on sustained attention performance, as measured by the RVIP task, or on smoking urge. Regardless of expectancy, nicotine administration produced greater ratings of smoking satisfaction and dizziness, less smoking craving, and improved performance on the RVIP. No interactions between the expectancy and drug manipulations were observed.

Our brief laboratory manipulation was effective in altering the expectancies of daily cigarette smokers, resulting in two groups that differed significantly in their expectancies for the effect of nicotine on performance, despite having similar expectancies before the manipulation. In terms of absolute change, those told that nicotine enhances performance reported a significant increase in nicotine performance expectancy, while those told nicotine impairs performance reported a decrease in nicotine performance expectancy that was not significant. Nicotine has been shown to enhance cognitive performance, and smokers appear to hold expectancies consistent with this effect. Thus, it is not too surprising that the expectancy manipulation had a stronger effect among individuals who were told that nicotine enhances performance compared to those who were told that nicotine impairs performance. This is consistent with the idea that expectancy change occurs most easily when instructions match participants' experience (Bolles, 1972; Christiansen, Goldman, & Inn, 1982; Kirsch & Lynn, 1999). This study, along with others, demonstrates that expectancy change

for smokers is possible in the laboratory and should be investigated further (Copeland & Brandon, 2000). If treatments and prevention campaigns incorporate expectancy challenges, further research is needed to determine how these challenges can be most effective.

Relative to those expecting impairment, participants expecting performance enhancement reported that the experimental cigarette, irrespective of its nicotine content, produced more psychological reward, more enjoyable physical sensations, and less craving. This is consistent with prior research showing that expectancy manipulations for one particular response (in this case, performance) may generalize to other responses (Harrell & Juliano, 2009). This may represent support for the memory network model in expectancy theory (Collins & Loftus, 1975; Goldman, Del Boca, & Darkes, 1999). This theory suggests that information is stored in "nodes" in memory and that a network of pathways links the information. Activation of one "node" leads to spreading activation of other information. Recent alcohol and tobacco research supports this theory (Linkovich-Kyle, Schreiner, & Dunn, 2012; O'Connor & Colder, 2009). However, it is important to note that there were no effects of the expectancy or nicotine manipulations on ratings of smoking urge. It is possible that the instructions to all participants that the cigarette contained nicotine, combined with the act of smoking itself, may have had a strong effect on urge, thereby overshadowing any potential effect of the response expectancy manipulation. Denicotinized cigarettes have been found to reduce deprivation-induced craving, at times similar to regular cigarettes (Barrett, 2010; Gross et al., 1997).

Motivation, broadly construed, has been suggested as a potential mechanism of placebo effects (Geers, Weiland, Kosbab, Landry, & Helfer, 2005; Hyland & Whalley, 2008). Consistent with prior research (Harrell & Juliano, 2009), we found that instructions that the drug enhances performance produced greater self-reported motivation for the RVIP task, but did not affect performance, potentially due to performance-based payment. Although not suggested by the authors, some published data has suggested that increases in motivation may be related to performance enhancement after placebo administration (Attwood, Terry, & Higgs, 2010). Further research is needed to explore the role of motivation in placebo effects to better understand how placebos affect health behaviors and other reactions, in both the short and the long term (Hyland, 2011).

We did not find the expected effect of the expectancy manipulation on actual cognitive performance. Two prior studies with caffeine found changes in motor performance consistent with the instructions, with those told enhance showing greater performance on the task (Fillmore et al., 1994; Fillmore & Vogel-Sprott, 1992). Of note, two other studies, one involving alcohol (Fillmore et al., 1994) and another involving caffeine users in withdrawal (Harrell & Juliano, 2009), found effects of the expectancy manipulation in the opposite direction, with those told impair actually performing better than those told enhance. Given the unique pharmacological properties of smoking and nicotine and the fact that there are no prior published studies that have assessed immediate reactions to smoking after directly manipulating smoking performance expectancies, it is difficult to explain the null findings in the present study. It appears that the expectancy manipulation was powerful enough to alter subjective outcomes but not the performance outcome. Although objective changes have been observed in response to placebo manipulations, most placebo research has focused on subjective effects. Future placebo research that includes both subjective and objective measures will improve our understanding of the full range of drug outcomes that are influenced by placebo processes.

Prior research has suggested that attempts to resist the impairing effects of the drug may account for improved performance among individuals led to expect that the drug will impair performance (Fillmore et al., 1994; Harrell & Juliano, 2009). There are a number of prior studies that have shown that resistance to drug effects, particularly alcohol, is associated with improved performance (Fillmore & Vogel-Sprott, 1995a, 1995b; Sdao-Jarvie & Vogel-Sprott, 1992) and, further, that instructions that interfere with resistance to drug effects can lead to impaired performance (Fillmore et al., 2002; Fillmore & Vogel-Sprott, 1994). This phenomenon is sometimes referred to as behavioral tolerance (Vogel-Sprott, 1997). It is possible that behavioral tolerance develops to withdrawal, similarly to how it develops to alcohol.

It is important to note that, due to nicotine abstinence instructions, the current study cannot discriminate nicotine withdrawal relief from nicotine performance enhancement. Nonetheless, consistent with prior research, nicotine administration led to greater smoking satisfaction and dizziness, and less craving (Juliano et al., 2011; Kelemen & Kaighobadi, 2007; Perkins et al., 2004). Furthermore, nicotine increased RVIP sensitivity (see Heishman et al., 2010, for a review). These findings are useful not only in providing additional data on the effects of nicotine in the context of smoking but also as a positive control by which to compare the magnitude of effects of the expectancy manipulation. Including pharmacological and psychosocial manipulations within the same study allows for a deeper understanding of both manipulations.

Study Limitations

The expectancy manipulation overall led to groups that had significantly different performance expectancies, but it was more effective in promoting nicotine performance enhancement expectancies. This may be due to participants' preexisting beliefs. Future research should explore ways of producing more powerful expectancy manipulations, especially when the information contradicts smokers' preexisting expectancies. Some research has noted sex differences with respect to the effects of nicotine instructions on cigarette smoking outcomes (Perkins et al., 2006). The disproportionate recruitment of men (70%) in the present study prohibits a powerful analysis of sex effects. All participants were led to believe that the cigarette contained nicotine, even though only 50% of participants actually received nicotine. Because additional control groups were not included, we do not know what impact stimulus expectancy or the simple act of smoking had on outcomes (Perkins et al., 2008; Perkins, Karelitz, Conklin, Sayette, & Giedgowd, 2010). Nevertheless, we were able to demonstrate that our expectancy manipulation had an effect beyond these factors on some of the measures.

Implications

A growing body of research supports the idea that drug effects are directly influenced by the expected outcomes of drug taking. This study demonstrated that performance expectancies of nicotine

could be experimentally manipulated and that expectancies influenced subjective reactions to smoking and task motivation. Clinically, this study provides further evidence that expectancy manipulations are possible and thus may be useful for treatment of nicotine dependence. These manipulations could take the form of expectancy challenges (Copeland & Brandon, 2000), advertisements, or hypnosis (Kirsch & Lynn, 1999). An important caveat to this advice is that expectancy manipulations appear to be most effective when they match a participant's experience. Thus, it may be more useful to point out the negative effects of cigarette smoking than to attempt to counter accurate beliefs about positive nicotine effects, such as cognitive enhancement. Rather than attempting to convince patients that nicotine impairs performance, treatment providers may want to challenge beliefs that nicotine is essential for adequate performance. Another strategy may be to note that nicotine withdrawal, which could be avoided with prolonged nicotine abstinence, is responsible for cognitive decrements. Future research should examine these possibilities. A greater understanding of the mechanisms underlying placebo effects, including response expectancy, will allow us to harness such effects to improve the overall effectiveness of pharmacological and psychological treatments.

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Received December 14, 2011
Revision received April 11, 2012
Accepted April 12, 2012 ■

Psychopharmacology
DOI 10.1007/s00213 012 2685 1

ORIGINAL INVESTIGATION

Transient compensatory smoking in response to placebo cigarettes

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Received: 27 October 2011 / Accepted: 27 February 2012
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Abstract
Rationale To address the public health problems caused by smoking, researchers have suggested a gradual reduction in the nicotine content of cigarettes. There remain concerns, however, about the potential for smokers to compensate for reductions in nicotine content by altering their smoking behavior. Such compensatory behaviors may negate any potential cessation and/or harm reduction benefits.
Objective The purpose of this study was to quantify smoking behavior (e.g., puff number, volume, duration, interpuff interval, and peak flow) in response to cigarettes, varying only in nicotine content, administered repeatedly.
Methods Sixty-seven dependent smokers participated in this two-session, within-subject study. Moderate nicotine content and placebo cigarettes (Quest© brand) were administered in a double-blind and counterbalanced manner. Each session required 12 h of tobacco abstinence and included four ad lib smoking bouts of the condition-assigned cigarette with 40 minutes separating each bout.
Results Placebo cigarettes produced increases in total puff volume and duration and decreases in total interpuff interval relative to cigarettes with moderate nicotine content. Differences in total puff volume and duration generally dissipated across smoking bouts, with differences in total puff volume nonexistent by the third and fourth bouts.
Conclusions Placebo cigarettes produce compensatory smoking during initial exposures; however, these effects appear to be short lived. These findings are consistent with the previous work where smoking compensation has been observed in response to a single cigarette, but not over several days of smoking.

Keywords Smoking · Topography · Compensation · Nicotine · Extinction

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Introduction

As an instrument for nicotine delivery, the cigarette is ideal for producing dependence. Inhaled nicotine is absorbed rapidly into the bloodstream and delivered to the brain within 7–10 s, with blood levels peaking within a few minutes (Benowitz 1996; Benowitz and Henningfield 1994; Hoffmann et al. 1997). The speed of nicotine delivery appears critical to the reinforcement of nicotine administration, with slower methods of delivery, such as buccal (gum) and transdermal (patch), generally recognized as less addictive than inhalation (i.e., smoking; Perkins 1999). Benowitz and Henningfield (1994) have proposed a threshold value of nicotine that a cigarette must contain to maintain dependent smoking. This threshold is based upon the estimated daily nicotine intake of minimally dependent smokers (4–6 mg daily) and the absolute bioavailability of the nicotine contained in cigarettes. In turn, it has been suggested that the reinforcing value of smoking behavior could be reduced or extinguished by limiting the nicotine delivered via cigarettes (Benowitz and Henningfield 1994; Walker et al. 2009).

In contrast to the hypothesis that reductions in nicotine will reduce smoking behavior, there is considerable evidence demonstrating that smokers compensate for these reductions by altering their smoking behavior (Scherer 1999). Smokers have been shown to take more frequent, larger, and/or longer puffs when they switch from higher (e.g., full flavor) to lower (e.g.,

Published online: 20 March 2012

light and ultralight) nicotine yield commercial cigarettes (Blank et al. 2009; Herning et al. 1981). Importantly, although full flavor and light cigarettes yield different levels of nicotine as measured by machine-based smoking, light cigarettes do not actually contain less nicotine than full flavor cigarettes. In fact, among typical brand cigarettes, the percentage of nicotine in tobacco has an inverse relationship with machine-measured nicotine yield (Benowitz et al. 1983). Light cigarettes produce reduced nicotine yields by diluting smoke with air drawn in through ventilation holes on the filter. However, if smokers block these ventilation holes and/or alter their puff topography, the nicotine dose delivered by these cigarettes may be increased. As such, the machine derived nicotine yield of consumer brand cigarettes does not predict blood levels of nicotine exposure biomarkers (Benowitz et al. 1983).

Moreover, reductions in nicotine yield achieved by smoke dilution are traditionally accompanied by reductions in other tobacco smoke constituents such as tar (see Federal Trade Commission 2000), which may also result in compensatory smoking behavior (Sutton et al. 1982). It remains unclear whether compensatory smoking patterns occur in response to reductions in nicotine, tar, or a combination of the compounds present in tobacco smoke. To provide a more stringent test of the effects of nicotine on smoking behavior, it is prudent to examine the effects of cigarettes, which vary in nicotine content, but produce similar levels of tar and other tobacco smoke constituents (Robinson et al. 2000). Well-suited for this purpose, Quest cigarettes (Vector Tobacco, Inc., Research Triangle Park, NC) are manufactured from tobacco that has been genetically modified to vary in nicotine content, but produce comparable levels of other smoke constituents (e.g., 10 mg tar). Specifically, Quest cigarettes are available with nicotine contents of 8.9 mg (Quest 1), 5.1 mg (Quest 2), and 1.0 mg (Quest 3) per cigarette, resulting in smoking machine-based nicotine yields of 0.6, 0.3, and <0.05 mg, respectively. Quest 3 cigarettes can be considered placebo cigarettes as their nicotine yield is negligible (Robinson et al. 2000).

Smokers have been administered all three nicotine doses in a counterbalanced order (30 min interbout interval), 30 min after smoking their own brand of cigarette ad libitum (Strasser et al. 2007). Larger puff volumes were observed for the placebo dose (Quest 3) relative to a moderate dose (Quest 2), but not the highest dose (Quest 1), providing partial evidence for compensatory smoking between single cigarette trials of varying nicotine content (see also Pickworth et al. 1999). Smoking topography across nicotine dose has also been investigated during extended periods of repeated exposure. Smokers given an unlimited supply of either placebo (Quest 3) or nicotine-containing (Quest 1) cigarettes over an 11-day period showed no changes in smoking topography (i.e., puff volume, puff duration, interpuff interval, peak flow, or average flow) between doses across three evaluation days (Donny et al. 2007). However, overall daily consumption decreased among those receiving placebo cigarettes, supporting the hypothesis that lower nicotine content cigarettes may reduce the reinforcement value of smoking (see also Buchhalter et al. 2005). A separate study found that individuals assigned to smoke only placebo cigarettes for a 7-day period actually displayed reduced total puff volumes relative to those receiving nicotine-containing cigarettes (Donny and Jones 2009). These findings appear to contradict earlier studies which have observed persistent compensatory smoking patterns when smokers switched from high-yield to lower-yield commercial cigarettes (as in Guyatt et al. 1989). Again, this discrepancy may be explained by differences in the amount of nicotine contained (as opposed to machine-derived nicotine yield) in the cigarettes investigated.

As with many other substances of abuse, preclinical studies investigating the self-administration of nicotine have consistently observed inverted *U*-shaped dose response patterns; self-administration rates increase and then decrease with increasing dose concentrations (Meisch 2000; Rose and Corrigall 1997; Fowler and Kenny 2011; Corrigall and Coen 1989; Donny et al. 1995; Risner and Goldberg 1983; Sannerud et al. 1994). Along the descending end of the curve, a reduction in dose concentration can result in an increased self-administration rate. This is consistent with the prolonged compensatory smoking patterns observed when smokers are switched from high-yield (full flavor) to lower-yield (light) cigarettes (Guyatt et al. 1989). In contrast, placebo doses should produce extinction of self-administration.

Indeed, extended use of placebo cigarettes has been shown to result in a gradual reduction in smoking behavior (Benowitz et al. 2007; Donny et al. 2007; Hatsukami et al. 2010a). A simulation study predicted that reduced nicotine content cigarettes could reduce national smoking rates to 5% and save 157 million quality-adjusted life years (Tengs et al. 2005). However, there is also evidence that individuals show acute compensatory smoking behaviors in response to these same cigarettes, potentially increasing harm from smoke exposure (Strasser et al. 2007). Given the limited number of studies using such cigarettes and the potential impact of this research for informing public policy (Hatsukami et al. 2010b), it is imperative to characterize the presence, magnitude, and duration of compensatory smoking in response to placebo cigarettes. Doing so will also help delineate the effects of total cigarette nicotine content on smoking behavior and reconcile human and animal investigations of nicotine self-administration.

The present study investigated the effects of nicotine content on smoking topography measures using experimental cigarettes which vary only in nicotine content. Within each of two sessions, dependent smokers' puff topography

was observed across four nicotine-containing (8.9 mg) or placebo (1.0 mg) cigarettes. Previous work suggests that compensatory smoking behavior would be observed in response to the placebo cigarette, relative to the nicotine-containing cigarette, and that the magnitude of compensatory smoking would diminish across cigarette trials.

Method

Participants

Eighty-three smokers were recruited from the Tampa Bay area for participation in a study investigating the effects of nicotine content on neural indices of attention (the primary study data are not reported here). Inclusion criteria were as follows: between the ages of 18–70 years and use of 15 or more cigarettes a day for the past 2 years (current smoking was biochemically verified; carbon monoxide level of ≥10 ppm, urinary cotinine level of ≥100 ng/mL). Participants were excluded if they used nicotine products other than cigarettes within the past 3 months; tested positive for psychoactive drug use (i.e., benzodiazepines, barbiturates, opiates, amphetamines, cocaine, PCP), tricyclic antidepressants, or pregnancy; reported any past head injury or loss of consciousness; had a current serious medical condition (e.g., cardiopulmonary problems, cancer, other major life-threatening illnesses); or were unable to read and understand consent forms and questionnaires. Participants were also administered the Structured Clinical Interview for DSM disorders (SCID; First et al. 1994) and were excluded if they met criteria for a DSM-IV Axis I disorder (i.e., psychosis, major depressive episode, manic/hypomanic episode, panic disorder, current alcohol, or substance dependence).

Sixteen participants were excluded from the present analysis because of procedural errors in the use of the smoking topography equipment that occurred during a 2-month period of time. Of the remaining sample of 67 participants (49 males), 54 identified themselves as white, 12 as black or African American, and 1 as an American Indian or Alaskan Native. The average age was 39.8 years old (range=19–63; SD=11.6); participants smoked 22.4 cigarettes per day (range=15–40; SD=6.7) and had a Fagerström Test of Nicotine Dependence (FTND; Heatherton et al. 1991) score of 5.74 (SD=1.85), indicative of moderate dependence on nicotine. The study was approved by the institutional review board of the University of South Florida and as such was conducted in accordance with the standards outlined in the 1964 Declaration of Helsinki.

Procedure

Following the completion of an assessment session during which informed consent was obtained, and eligibility was established, participants completed two separate 2.5-h experimental sessions (scheduled 3 to 14 days apart). Sessions were double-blinded, counterbalanced, and only differed in the nicotine content of the cigarettes administered: Quest 1 (8.9 mg) or Quest 3 (1.0 mg) cigarettes (Vector Tobacco Inc., Research Triangle Park, NC). To standardize presession tobacco and alcohol consumption, 12-h abstinence from smoking (CO level of <10 ppm or no greater than half of their CO level at initial assessment) and alcohol (blood alcohol level of <.001%) was required. Participants who complied with these restrictions then smoked the first of four cigarettes at their own pace through a mouthpiece connected to a smoking topography device (they were told that cigarettes would contain varying doses of nicotine). Each subsequent cigarette was smoked 40 min following initiation of the previous cigarette, and all cigarettes were followed immediately by completion of a subjective questionnaire (described below). Participants were prepped with an electroencephalogram (EEG) cap between smoking bouts 1 and 2, and administered attention and working memory tasks between smoking bouts 2 and 3, and bouts 3 and 4 (data to be reported elsewhere).

Measures

Smoking topography measures included total puff volume, total puff duration, total interpuff interval, total number of puffs, and average maximum peak air velocity per cigarette. These data were measured using the Clinical Research Support System (CReSS; Borgwaldt, KC), a computerized device shown to have negligible effects on smoking behavior and to be effective for quantifying smoke exposure (Blank et al. 2009; Lee et al. 2004).

The subjective questionnaire administered after each smoking bout was the Modified Cigarette Evaluation Questionnaire (mCEQ; Cappelleri et al. 2007). The mCEQ contains 12 items and assesses five domains: smoking satisfaction, psychological reward, aversion (e.g., dizziness and nausea), sensory feelings, and reduction in craving.

Data analyses

To examine compensatory effects, we assessed nicotine effects (nicotine vs. placebo) across the four cigarettes via mixed-model repeated measures analyses. The models included effects for nicotine content (nicotine vs. placebo), cigarette trial (1, 2, 3, and 4), and the interaction of these two factors as fixed effects, with cigarette trial as a random effect. Primary dependent variables included all topography values mentioned above. For each measure, values for the first puff, which occurred directly after lighting, were omitted. Subjective responses to smoking were also examined with mixed-model analyses. Planned comparisons of

nicotine content, across each smoking bout, were conducted when mixed-models revealed significant main effects that included nicotine content (i.e., nicotine content or nicotine content X cigarette trial interaction).

Results

Smoking topography

Figure 1 shows the means for topography measures for each cigarette smoked during the two sessions. For total puff volume and total puff duration (Fig. 1a, b), there were significant effects for nicotine content [$F(1, 74)=5.8$ and $F(1, 76)=20.2$, p's≤ 0.02], as well as a significant nicotine content X cigarette trial interaction [$F(3, 140)=2.9$ and $F(3, 143)=3.4$, p's<0.05]. This indicates that there were differential changes in puff volume and duration across cigarette trials, as a function of nicotine content. Specifically, participants produced significantly elevated puff volumes, while smoking the placebo cigarettes during the first [$F(1, 77)=4.4$, $p=0.04$] and second [$F(1, 75)=13.3$, $p<0.001$] smoking bouts, but not during the remaining smoking bouts (p's>0.33). A similar pattern was apparent for puff duration [first bout, $F(1, 79)=14.5$, $p<0.001$; second bout, $F(1, 75)=29.5$, $p<0.001$], except that the difference between the lowest and highest nicotine content during the final cigarette trial was also significant, $F(1, 68)=5.2$, $p=0.03$. Total interpuff interval (Fig. 1c) was significantly reduced with placebo cigarettes [$F(1, 66)=28.2$, $p<0.001$]; however, no effects were evident for the cigarette trial or the interaction term (p's>0.29). Thus, there were significantly longer intervals between puffs for nicotine-containing cigarettes, across all four bouts (p's≤ 0.03). Number of puffs taken and average peak air velocity (Fig. 1d, e) showed no significant effects for nicotine content (p's>0.07), cigarette trial (p's>0.50), or their interaction (p's>0.12).

Subjective effects

Figure 2 depicts the subjective ratings for each cigarette smoked during the two sessions. Smoking satisfaction and sensory feelings were greater in the nicotine condition [$F(1, 76)=12.9$ and $F(1, 75)=11.8$, respectively, p's$=0.001$]. These subjective ratings also showed reduction across cigarette trial [$F(3, 271)=5.0$ and $F(3, 263)=7.7$, respectively, p's≤ 0.002], but no interactions between nicotine and cigarette trial were observed (Fig. 2a, b; p's>0.21). Thus, participants rated cigarettes with higher nicotine content as more satisfying and indicated that they enjoyed the sensory aspects to a higher degree than when smoking cigarettes with lower nicotine content; these effects diminished across smoking trials, regardless of nicotine content. The nicotine-containing cigarettes were also found to reduce cravings to a greater extent than placebo cigarettes [Fig. 2c; $F(1, 89)=10.3$, $p=0.002$)]. In addition, a statistical trend for the interaction between cigarette trial and nicotine content indicated that craving reduction occurred following the first cigarette trial, but was attenuated thereafter, $F(3, 150)=2.5$, $p=0.07$. Differences between nicotine content were significant only during the first cigarette trial [$F(1, 74)=4.0$, $p<0.001$], and approached significance for the second bout, $F(1, 73)=1.2$, $p=0.05$. Finally, psychological reward and aversion ratings were significantly affected by nicotine content [$F(1, 65)=9.1$ and $F(1, 85)=27.3$, p's≤ 0.004], cigarette trial [$F(3, 219)=5.6$ and $F(3, 237)=15.9$, p's≤ 0.001], and their interaction [$F(3, 117)=3.1$ and $F(3, 118)=3.3$, p's<0.05] (Fig. 2d, e), that is, smoking was generally more rewarding and aversive (i.e., caused a higher degree of dizziness and nausea) for higher dose cigarettes, but these differences attenuated across bouts.

Discussion

The aim of this study was to examine the time course of compensatory smoking behavior by comparing smoking topography responses to multiple nicotine-containing (8.9 mg) and placebo (1.0 mg) cigarettes smoked during two separate sessions. This sample of dependent smokers demonstrated compensatory behavior while smoking placebo cigarettes, but these effects generally dissipated across cigarette trials. For total puff volume, differences between nicotine-containing and placebo cigarettes were no longer detectable by the third cigarette smoked. For total puff duration and total IPI, some significant differences between conditions were still observable at the third and/or fourth smoking bouts, though they were markedly less pronounced than those observed at the first and second smoking bouts. For example, the absolute mean difference between nicotine and placebo for total puff duration was 2.5 s (SE=0.65), 3.1 s (SE=0.56), 1.2 s (SE=0.70), and 1.5 s (SE=0.66) for bouts 1, 2, 3, and 4, respectively. The differences in total puff duration at these later bouts were insufficient to produce significant elevations in total puff volume.

This pattern of results appears to differ from that of a previous work where exclusive use of placebo cigarettes reduced smoking behavior relative to otherwise identical nicotine-containing cigarettes (Donny et al. 2007; Hatsukami et al. 2010a). Of course, that smoking behavior diminished in the absence of nicotine was not unexpected, given that nicotine is considered the agent primarily responsible for maintaining smoking. However, increased self-administration responding is sometimes observed when dependent smokers are required to respond for placebo or have the effects of nicotine blocked by antagonist drugs (Rose and Corrigall 1997). Such compensatory responses are

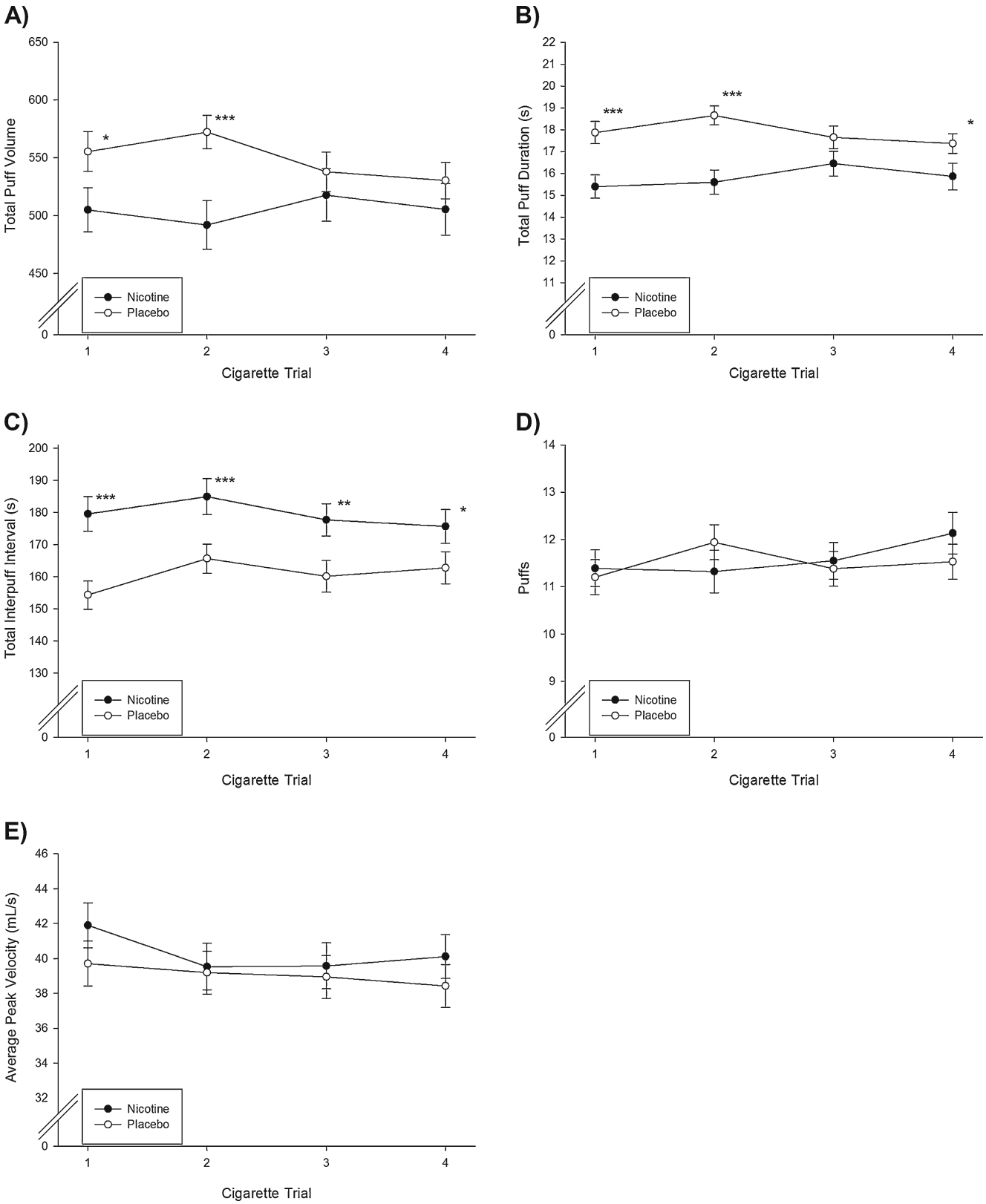


Fig. 1 Comparison of nicotine (*solid circles*) versus placebo (*open circles*) Quest cigarettes across sessions on: (**a**) total puff volume, (**b**) total puff duration, (**c**) total interpuff interval, (**d**) number of puffs, and (**e**) average maximum peak air velocity. *Error bars* indicate standard error. *$p<0.05$; **$p<0.01$; ***$p<0.001$

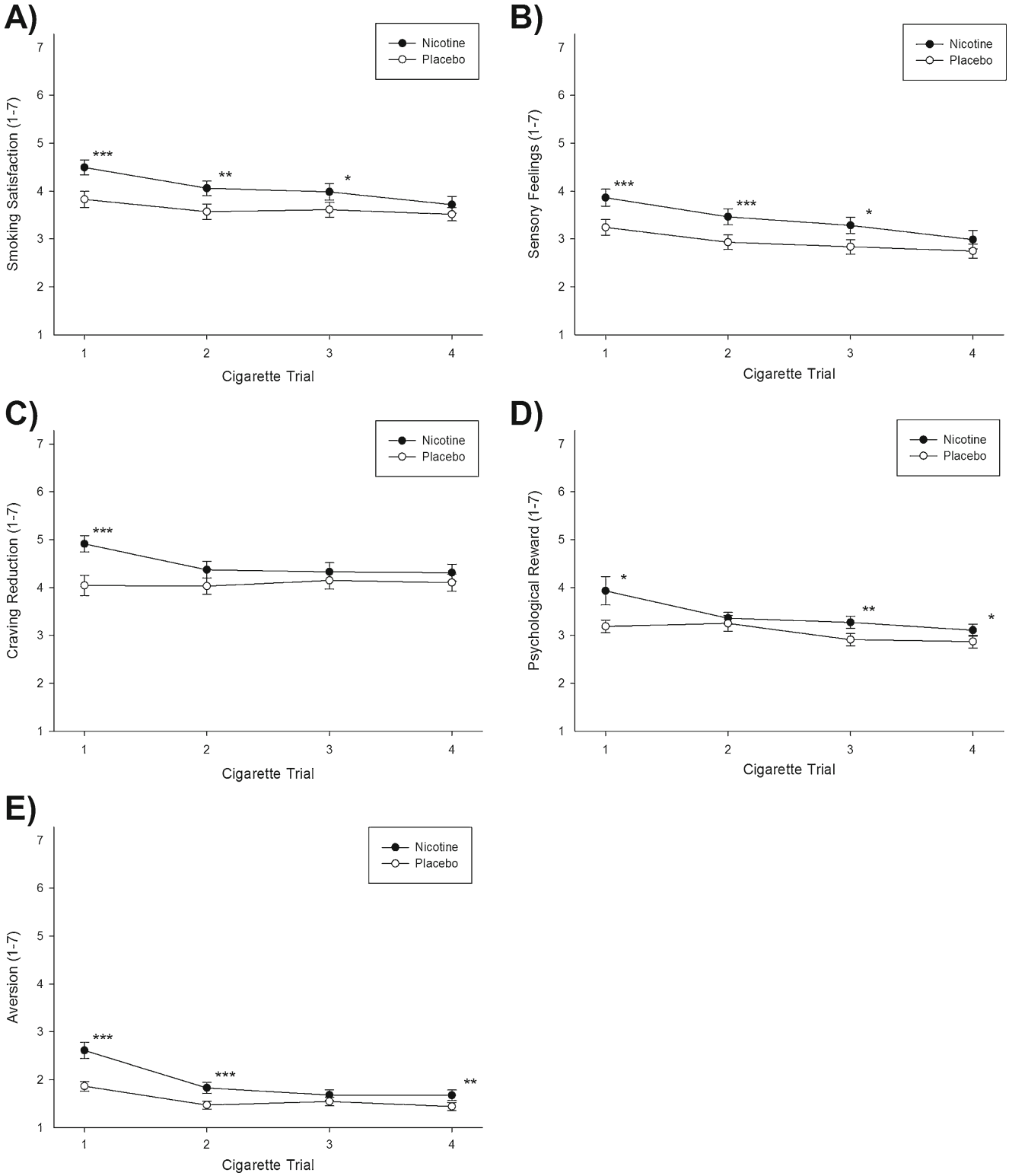


Fig. 2 Comparison of nicotine (*solid circles*) versus placebo (*open circles*) Quest cigarettes across sessions on mCEQ domains: (**a**) smoking satisfaction, (**b**) sensory feelings, (**c**) craving reduction, (**d**) psychological reward, and (**e**) aversion. *Error bars* indicate standard error. *$p<0.05$; **$p<0.01$; ***$p<0.001$

short lived and resemble extinction bursts of the sort observed in humans and animals when reinforcer delivery is suspended.

Extinction burst responding has typically not been observed in animal investigations of nicotine self-administration, though such an effect has been recently documented in rodents (Harris

et al. 2007). Substitution of nicotine with saline after the establishment of nicotine self-administration produced increases in the rate of self-administration. However, these extinction bursts were very short lived (observable only during a period of an hour or two), and the magnitude and timing of extinction bursts was variable between subjects. This is consistent with our own data and suggests that the subtle and short-lived nature of these responses may make them difficult to detect with more distal measures of nicotine self-administration (e.g., cigarettes per day). Interestingly, in rodents, the production of an extinction burst was predicted using the rate of self-administration during the first two hours of drug availability during maintenance, and burst responding subsequently predicted resistance to extinction. As a proximal measure of nicotine administration, acute compensatory responses to placebo cigarettes may have the utility for predicting treatment responses in humans as well. Other topography measures have already been determined to predict cessation outcomes in adolescents (Franken et al. 2006) and adults (Strasser et al. 2004).

One might also consider the present findings in the context of the response cost of drug-seeking behavior. When the nicotine yield of a cigarette is reduced via design features (i.e., ventilation), the user must smoke more intensively to deliver a nicotine dose comparable to that which could be obtained through less intensive smoking of a higher-yield cigarette. In both humans and animals, the self-administration of drugs can be maintained when the response cost is increased. However, the persistence of self-administration in the context of increased response cost is dose-dependent, that is, persistent responding is maintained when the response cost is increased at higher doses, but is not maintained at low doses (Meisch 2000). Thus, increased responding may be maintained when dependent smokers switch to commercial lower-yield cigarettes (which contain considerable nicotine), but not when switched to reduced nicotine content cigarettes. Because content is limited, compensation strategies that are employed while smoking these cigarettes do not produce notable increases in nicotine delivery (Benowitz et al. 2006).

It is important to note, however, that most smokers do not completely cease smoking even when restricted to placebo cigarettes (Hatsukami et al. 2010a). Furthermore, smokers often consider placebo cigarettes as more aversive (e.g., increased ratings of "harsh," decreased ratings of "taste good") than their usual brand (Buchhalter et al. 2005; Donny et al. 2007), which may influence natural smoking behavior. In the current study, ratings for aversion were generally low for both types of Quest cigarettes. Although the nicotine-containing cigarettes were rated as more aversive than the placebo dose initially, these differences also dissipated over the repeated smoking bouts. Taken together, current and previous work suggest the need for additional research to validate the idea that placebo cigarettes are unlikely to maintain self-administration behavior, and thus may be effective as a smoking cessation aide (see Hatsukami et al. 2010b; Walker et al. 2009). Future studies using multiple doses of nicotine will be necessary to determine if there is indeed a threshold dose of nicotine content necessary to maintain regular smoking as proposed by Benowitz and Henningfield (1994). Fortunately, a new line of reduced nicotine cigarettes are currently being tested for research purposes through the National Institute of Drug Abuse. Several doses of nicotine will be offered, including doses that fall below those previously available with Quest (i.e., 0.02 mg).

To confirm that the compensatory patterns described presently reflect extinction burst responding, it will be important for future studies to demonstrate that short-lived compensation emerges when dependent smokers are switched to placebo cigarettes after being maintained on otherwise identical nicotine-containing cigarettes. Comparisons with conventional brand smoking should be avoided, as these traditional cigarettes also vary in other ingredients and design features likely to affect smoking topography. The present study tested smokers after 12 h of smoking abstinence in order to standardize presession nicotine exposure. Sessions began in the morning hours, and thus the present findings may be most relevant to the effects of nicotine content after overnight abstinence. Future research should determine whether these same effects are observed after shorter periods of abstinence. Additionally, larger sample sizes will be necessary to detect moderators of compensation. For example, genetic polymorphisms (e.g., Strasser et al. 2011) or assessments of dependence (e.g., Brauer et al. 2001) may predict those more or less likely to compensate and/or to extinguish smoking behavior with reduced nicotine cigarettes.

In summary, the present results are consistent with prior observations of smoking compensation in response to placebo (Quest 3) versus nicotine-containing (Quest 2) cigarettes during a single smoking trial (Strasser et al. 2007). Furthermore, we provide evidence that compensatory smoking in response to placebo cigarettes partially extinguishes over the course of several cigarettes. These findings appear to be consistent with a lack of compensation when smokers are observed across multiple days of testing (Donny et al. 2007) and suggest that compensatory smoking may not pose a significant limitation to the use of low-nicotine cigarettes for cessation and harm reduction efforts. However, these results should be replicated and extended before being applied to smoking cessation and harm reduction treatment protocols.

Acknowledgments This study was funded by NIH grants R21 DA027001, R21 DA024226 and R25 CA090314. The authors would like to thank Renee Ornduff and Natasha Garcia for their work on the project.

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Pharmacology, Biochemistry and Behavior xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Nicotine content and abstinence state have different effects on subjective ratings of positive versus negative reinforcement from smoking☆

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ARTICLE INFO

Article history:
Received 16 February 2012
Received in revised form 21 November 2012
Accepted 24 November 2012
Available online xxxx

Keywords:
Nicotine
Smoking
Placebo
Abstinence
Subjective ratings
Conditioned reinforcement

ABSTRACT

Despite the well known adverse health consequences of smoking, approximately 20% of US adults smoke tobacco cigarettes. Much of the research on smoking reinforcement and the maintenance of tobacco smoking behavior have focused on nicotine; however, a number of other non nicotine factors are likely to influence the reinforcing effects of smoked tobacco. A growing number of studies suggest that non nicotine factors, through many pairings with nicotine, are partially responsible for the reinforcing effect of smoking. Additionally, both clinical studies and preclinical advances in our understanding of nicotinic receptor regulation suggest that abstinence from smoking may influence smoking reinforcement. These experiments were conducted for 2 reasons: to validate a MRI compatible cigarette smoking device; and to simultaneously investigate the impact of nicotine, smoking associated conditioned reinforcers, and smoking abstinence state on subjective ratings of smoking reinforcement. Participants smoked nicotine and placebo cigarettes through an fMRI compatible device in an overnight abstinent state or in a nonabstinent state, after having smoked a cigarette 25 minutes prior. Outcome measures were within subject changes in physiology and subjective ratings of craving and drug effect during the smoking of nicotine or placebo cigarettes on different days in both abstinence states. Cigarette type (nicotine vs. placebo) had a significant effect on positive subjective ratings of smoking reinforcement ("High", "Like Drug", "Feel Drug"; nicotine > placebo). In contrast, abstinence state was found to have significant effects on both positive and negative ratings of smoking reinforcement ("Crave", "Anxiety", "Irritability"; abstinence > nonabstinence). Interaction effects between abstinence and nicotine provide clues about the importance of neuroadaptive mechanisms operating in dependence, as well as the impact of conditioned reinforcement on subjective ratings of smoking induced high.

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1. Introduction

Cigarette smoking was the leading preventable cause of death in the US in 2010 (CDC, 2010) with approximately 19.3% of US adults self identifying as cigarette smokers (CDC, 2008). About 443,000 people die annually due to tobacco related causes and more than $190 billion is spent annually on health, productivity, and other tobacco related costs (CDC, 2009). Despite the well known risks of illness and death from smoking, and the availability of numerous alternative routes of nicotine self administration, people continue to smoke.

Nicotine is the primary psychoactive constituent of tobacco cigarettes (for review see Henningfield and Fant, 1999), and nicotine is certainly one factor that contributes to cigarette smoking. However, the reinforcing efficacy of nicotine, as measured using a variety of commonly used laboratory paradigms, is modest compared to that of other abused psychostimulant drugs such as cocaine and the amphetamines. A number of lines of evidence suggest that the conditioned reinforcers associated with drug self administration may play a larger role in addiction to tobacco compared to other drugs (Buchhalter et al., 2005; Caggiula et al., 2001, 2002; Le Foll and Goldberg, 2005; Naqvi and Bechara, 2005, 2006; Perkins et al., 2003), and additional papers report a critical role of smoking related conditioned reinforcers in maintenance of cigarette dependence (Lazev et al., 1999; Rose, 2006; Rose et al., 2000, 2003, 2010). Reinforcement from smoking likely occurs as the result of a combination

☆ The authors have no financial interests to disclose concerning the conduct or reporting of this study. This research was supported by NIDA grants K01DA021730 and R03DA021231 (KPL), K25DA14013 (BBF), K05DA00343 and T32 DA15036 (SEL).

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0091-3057/$ – see front matter © 2012 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.pbb.2012.11.012

Please cite this article as: Lindsey KP, et al, Nicotine content and abstinence state have different effects on subjective ratings of positive versus negative reinforcement from smoking, Pharmacol Biochem Behav (2012), http://dx.doi.org/10.1016/j.pbb.2012.11.012

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of factors including both the pharmacological effects of nicotine on the central and peripheral nervous system (Lee et al., 1993; Naqvi and Bechara, 2005; U.S.D.H.H.S., 1988), and non nicotine sensory aspects of smoking. For example, the taste and smell of smoke and the sensation of smoke in the throat and lungs have previously been shown to be important for the subjective effects of cigarette smoking (Perkins, 1999), and have also been shown to be conditioned to be reinforcing themselves through repeated pairings with nicotine (Rose, 2006; Rose et al., 2000, 2003; Rose and Levin, 1991).

Nicotine free cigarettes, whether manufactured from de nicotinized tobacco or from genetically modified "nicotine free" (0.03 mg/nicotine/cigarette) tobacco, provides an opportunity to evaluate smoking associated conditioned reinforcers de coupled from nicotine delivery. Previous results indicate that placebo cigarettes are surprisingly reinforcing. Nicotine free cigarette smoking robustly reduces craving (Butschky et al., 1995; Donny et al., 2007; Gross et al., 1997; Pickworth et al., 1996) and produces similar ratings of satisfaction compared to cigarettes containing pharmacologically active doses of nicotine (Brauer et al., 2001; Butschky et al., 1995; Pickworth et al., 1996). Within subject comparisons of the subjective reinforcement produced by smoking nicotine containing versus placebo cigarettes allow separation of the effects of nicotine from the effects of conditioned reinforcers.

Smoking related reinforcement is also likely to depend on several other factors relating to the regulation of signaling via nicotinic acetylcholine receptors (nAChRs). Experimental results from several model systems ranging from transfected xenopus oocytes to post mortem autoradiography in human brain suggest that nicotine causes not only nAChR activation, but also upregulation of nAChRs and both short term and long term desensitization of nAChRs (reviewed by Govind et al., 2009; Picciotto et al., 2008). The timing of cigarette administrations in these experiments was chosen in order to measure changes in subjective effects of smoking in a receptor sensitized (after overnight abstinence) and receptor desensitized state (30 minutes after the first cigarette) in smokers.

Two sets of experiments were conducted to separate the impact of cigarette type and smoking abstinence state on subjective ratings of reinforcement from smoking. Both cigarette type and abstinence state were hypothesized to have significant effects on subjective ratings of nicotine's effect with the nicotine condition and the abstinent condition producing the greatest changes in ratings. We also hypothesized that the effects of abstinence might differ depending on cigarette type with greater impact of abstinence observed in the nicotine condition. In the first experiment, an fMRI compatible smoking device was developed and validated in a study of nine smokers outside the scanner. In the second experiment, 14 cigarette smokers, smoked two nicotine cigarettes through the device (Lindsey et al., 2009) during one visit, and two placebo cigarettes thorough this device on a separate visit (blind to cigarette type) while providing subjective ratings of smoking effect during concurrent fMRI scanning (imaging data to be reported elsewhere). To our knowledge, this study is the first to systematically investigate whether responses to nicotine and placebo cigarettes differ during smoking in an abstinent versus a non abstinent state.

2. Materials and methods

2.1. Participants

Nine smokers, 78% male ($n=7$) and 22% female ($n=2$) with an average age of 27.1 years (SD = 7.9 years), completed the device validation study. The average Fagerstrom Test for Nicotine Dependence (FTND) score in this group was 4.8 (SD = 2.4). The average number of cigarettes smoked per day was 16.7 (SD = 10.1).

Fourteen smokers, 64% female ($n=9$) and 36% male ($n=5$) with an average age of 29.4 years (SD = 8.1 years), completed all four smoking conditions in the fMRI scanner. The average FTND in this group was 3.6 (SD = 3.3). The average number of cigarettes smoked per day was 15.0 (SD = 8.5).

Smokers were recruited from the community through online ads on craigslist.org. After a brief initial phone questionnaire, qualified participants were brought to the Behavioral Pharmacology Research Laboratory at McLean Hospital for a screening visit. Participants read and signed an Institutional Review Board approved informed consent form, filled out a number of questionnaires on smoking history, previous drug use, medical history, and an MRI compatibility questionnaire (only in the fourteen scanned participants) to ensure that no subject had any contraindicating condition. Participants from both studies completed a physical examination to ensure participants were in good physical health (EKG, complete blood panel, negative drug urine screen) (QuickTox Drug Screen Dipcard, Branan Medical Corporation, Irvine, CA), were in good mental health (no axis I disorders revealed by the Structured Clinical Interview and Diagnostic tool (SCID; (First et al., 2002)), and were not pregnant (Stanbio QuPID One Step Pregnancy Test Procedure No. 1220, Studio Laboratory, Boerne, Texas)). Nicotine dependence was assessed using the Revised FTND (Heatherton et al., 1991). Participants who met all screening criteria returned to the lab for the experimental visits.

2.2. Experimental procedures

2.2.1. Experiment 1: device validation

Two separate experiments were performed; the first was conducted to validate the ability of the MRI compatible smoking device to deliver nicotine and produce a subjective smoking experience similar to that produced by conventional cigarette smoking by hand. Nine device validation participants completed two experiments in the psychopharmacology laboratory. During one visit they smoked a nicotine containing cigarette by hand in the normal fashion, and during the other visit they smoked a nicotine containing cigarette through the MRI compatible device (Frederick et al., 2007), modified for improved drug delivery (Lindsey et al., 2009). The order of the experimental visits was counterbalanced. Each smoking session included a 5 minute baseline period, a 5 minute paced smoking period (1 puff every 21 seconds), and a 15 minute post smoking period.

Breath CO (Vitalograph CO meter, Lenexa, KS) was measured before and after smoking. Heart rate was monitored at one second intervals while blood pressure was taken at 3 minute intervals using a Welch Allyn Model 6200 Patient Monitor (Beaverton, OR), and blood samples for serum nicotine quantification were taken at 2 or 4 minute intervals (Harvard Apparatus Infusion Model 901, S. Natick, MA). During the entire experiment, a subjective ratings questionnaire was administered every 2.5 minutes using a computerized visual analog scale (using Presentation Software Version 0.92, Neurobehavioral Systems, Inc., Albany, CA). A handheld fiber optic response panel (Current Designs, Philadelphia, PA) allowed participants to report a time course of subjective ratings of drug effects. A questionnaire was designed to acquire data on changes in positive and negative reinforcement during the experiment. Seven unique questions were asked: "How HIGH do you feel right now?", "How much do you CRAVE a cigarette right now?", "How much do you LIKE the DRUG that is in your body right now?", "How IRRITABLE do you feel right now?", "How much do you FEEL DRUG EFFECT right now?", and "How ANXIOUS do you feel right now?". These questions were selected to gather information about both positive reinforcement (HIGH, LIKE DRUG, FEEL DRUG) and negative reinforcement (CRAVE, IRRITABLE, ANXIOUS) from smoking. Blood samples were allowed to clot then serum was separated by centrifugation. Serum nicotine was quantified by National Medical Services, Inc. (Willow Grove, PA) by LC MS/MS.

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Please cite this article as: Lindsey KP, et al, Nicotine content and abstinence state have different effects on subjective ratings of positive versus negative reinforcement from smoking, Pharmacol Biochem Behav (2012), http://dx.doi.org/10.1016/j.pbb.2012.11.012

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2.2.2. Experiment 2: investigation of cigarette type and abstinence state

Fourteen additional participants were studied on two separate days (one placebo cigarette visit and one nicotine cigarette visit, in counterbalanced order) and smoked two cigarettes through the MRI compatible smoking device with concurrent fMRI scanning (using a Siemens Trio 3 Tesla whole body MR scanner, using a transmit receive quadrature birdcage head coil). On fMRI scanning visits, expired CO was tested to verify abstinence from smoking (at least 10 hours, breath CO less than 15 ppm), and a urine screen was performed to verify that participants tested negative for illicit drugs (and pregnancy in females). Each smoking session included fMRI and subjective data collection during a five minute baseline period, a five minute paced (1 puff every 21 seconds) air puffing period during which participants puffed on the unlit cigarette, a five minute period of paced cigarette smoking (1 puff every 21 seconds); functional scanning continued dur ing a 10 minute post smoking period. The smoking session lasted for 25 minutes total. After the end of the post smoking period, participants had 5 minutes of additional scanning without subjective ratings ques tionnaires before the entire 25 minute smoking procedure outlined above was immediately repeated with the same cigarette type as the previous scan (in a non abstinent state). Each participant completed two fMRI scanning visits on separate days, which were identical except that during one visit two nicotine containing cigarettes were smoked (Marlboro "Red" 100 s; 1.1 mg nicotine per cigarette machine smoked yield), and on the other visit two non nicotine placebo cigarettes were smoked (Quest 3 brand cigarettes; <0.05 mg nicotine per cigarette machine smoked yield). Aside from nicotine content, these two types of cigarettes are substantially similar; the best available evidence suggests that non nicotine alkaloids are present in approximately the same concentrations in the genetically modified Vector 21 41 tobacco strain used to manufacture Quest 3 (Xie et al., 2004) cigarettes compared to commercial Marlboro cigarettes (Wu et al., 2002) (Nornicotine: 400 ± 100 ppm in Vector 21 41 compared to 750 ± 10 ppm in Marlboro; other alkaloids: 460 ± 210 ppm in Vector 21 41 compared to 621 ± 120 ppm in Marlboro). For comparison, nicotine levels are 1440 ± 660 in Vector 21 41 compared to 18100 ± 1500 ppm in Marlboro.

2.3. Analysis

2.3.1. Experiment 1: Device Validation

The impact of the smoking device on the time courses of smoking related subjective ratings, changes in physiological mea sures, and plasma nicotine levels, was assessed using repeated mea sures ANOVAs (one for each outcome) with smoking condition (by hand or by device) and time as within subject factors, and serum nicotine, heart rate, blood pressure, and subjective ratings as out comes. For each subjective ratings time point, variance existing in participants before the experimental manipulation was normalized by subtracting baseline ratings (the average of the subjective ratings collected during the 5 minute baseline period that occurred at the beginning of each experiment). A Bonferroni adjustment was applied to pairwise comparisons between each time point and baseline (8 comparisons) for each outcome separately (unnormalized data shown in Fig. 1).

The impact of fMRI scanning on smoking related subjective rat ings and physiological changes was assessed using repeated measures ANOVAs (one for each outcome) with time as a within subject factor, and smoking condition (through the device in the lab or through the device in the scanner) as a between subject factor, and heart rate, and normalized subjective ratings as outcomes. Bonferroni adjustments were made for both condition and time variables for each outcome separately.

2.3.2. Experiment 2: investigation of cigarette type and abstinence state

Changes in subjective ratings of reinforcement over the entire time courses of all subjective ratings were also assessed with repeated measures ANOVAs, with cigarette type (nicotine or placebo), smoking abstinence state (smoking abstinent or not smoking abstinent), and time as within subject factors. For each outcome separately, to adjust for multiple comparisons, Bonferroni adjustments were made on cigarette type, abstinence state, and time variables (Fig. 2).

In order to better understand the impact of conditioned rein forcers, subjective ratings data obtained at the time points that occurred during smoking (10, 12.5, and 15 minute time points were examined separately (Fig. 3)). During these time points, participants actively experienced smoking related conditioned reinforcers such as the taste and smell of smoke (either with or without nicotine). Data for each rating in each participant were also normalized to base line in order to reduce the impact of natural variability in subjective ratings between participants in the baseline state. Repeated measures ANOVAs were performed on these normalized smoking time points with cigarette type, abstinence state, and time as within subject fac tors. Bonferroni adjustments for multiple comparisons were made on cigarette type, abstinence state, and time variables separately for each outcome.

3. Results and discussion

3.1. Device validation

An initial set of experiments was conducted to determine whether smoking through the MRI compatible device delivered the same amount of nicotine, and produced the same physiological changes, and the same subjective ratings of drug effect as those produced by normal cigarette smoking without the device. No significant dif ferences were observed between normal by hand smoking and with device smoking for any outcome measure including plasma nicotine levels (Fig. 1A, $F(1,7) = 0.304$, $p = 0.599$), subjective ratings of nicotine effects ("High" $F(1,8) = 0.408$, $p = 0.541$, Fig. 1C; "Crave" $F(1,8) = 0.975$, $p = 0.352$, Fig. 1D), or physiological measures includ ing heart rate ($F(1,7) < 0.001$, $p = 0.989$, Fig. 1B), systolic blood pres sure (data not shown; $F(1,7) = 0.001$, $p = 0.983$), and diastolic blood pressure (data not shown; $F(1,7) = 0.432$, $p = 0.530$). Regardless of whether participants smoked through the MRI compatible device in the lab or smoked a cigarette by hand in the lab, cigarette smoking produced the same changes in plasma nicotine, subjective ratings, and physiological measures.

To determine whether MRI scanning influenced nicotine delivery, physiological changes, or subjective ratings of drug effect, outcome measures from sessions where nicotine cigarettes were smoked with the MRI compatible smoking device in the scanner were com pared to outcomes from sessions where cigarettes were smoked out side of the scanning environment. Subsequent analyses of the entire time courses of subjective ratings data collected in all participants during with device smoking and concurrent fMRI scanning showed an expected significant main effect of time for each rating. No sig nificant differences were observed in heart rate ($F(1,17) = 0.688$, $p = 0.418$, Fig. 1B) or subjective ratings of nicotine effects ("High" $F(1,21) = 0.072$, $p = 0.792$, Fig. 1C; "Crave" $F(1,21) = 0.135$, $p = 0.716$, Fig. 1D), regardless of whether participant smoked through the MRI compatible device in the lab or through the MRI compatible device during fMRI scanning. Together these data indicate that neither the smoking device, nor the scan had any significant effects on any outcome measure tested.

3.2. Impact of cigarette type and abstinence state on the time courses of ratings of positive reinforcement from smoking

A second series of experiments was conducted to assess the impact of cigarette type and abstinence on the response to smoking. Participants reported subjective ratings during 5 minutes of baseline, 5 minutes of paced air puffing, 5 minutes of paced smoking (one puff

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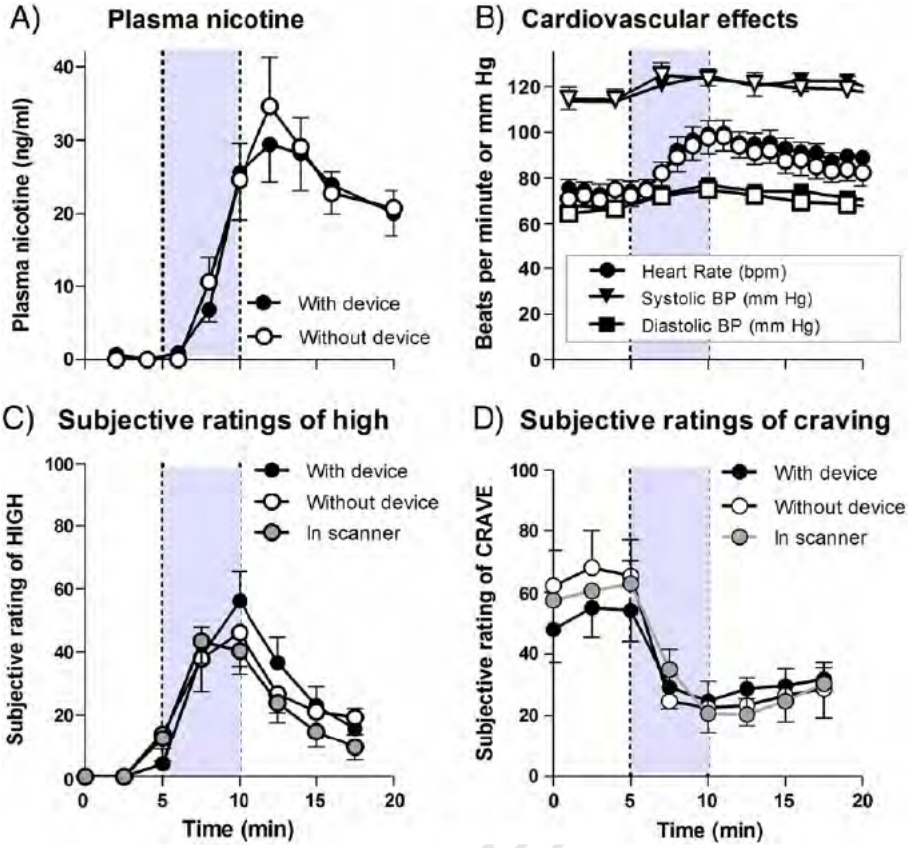


Fig. 1. Device validation. Smoking through the fMRI compatible device induced the same physiological changes and subjective ratings as a cigarette smoked by hand. The smoking period is shown in gray. (A) Increases in serum nicotine were not significantly different when a cigarette was smoked by hand (open circles) or through the device in the lab (closed circles). (B) Heart rate increase was the same regardless of whether the cigarette was smoked by hand (open circles), through the device in the lab (closed circles), or through the device in the scanner (gray circles). (C) Increases in ratings of "High" were not significantly different regardless of whether the cigarette was smoked by hand (open circles), through the device in the lab (closed circles), or through the device in the scanner (gray circles). (D) Decreases in ratings of "Crave" were also not significantly different regardless of whether the cigarette was smoked by hand (open circles), through the device in the lab (closed circles), or through the device in the scanner (gray circles). (Significance from baseline time-point: $^*p<0.05$). Note that these data are shown unnormalized in order to show the variance in craving between groups at baseline.

every 21 seconds) and for 10 minutes post smoking. In order to understand how these ratings evolved over time, the initial analysis considered the entire raw time course of each of six subjective ratings. A significant main effect of time was found for each of the three positive subjective ratings ("High", $F(1,9)=32.325$, $p\leq0.001$, Fig. 2A; "Feel Drug Effects", $F(1,9)=25.998$, $p=p\leq0.001$, Fig. 2B; "Like Drug", $F(1,9)=35.552$, $p\leq0.001$, Fig. 2C). Increases in positive ratings were present for smoking in *every* condition, regardless of the presence of nicotine. On average, for all four smoking conditions, the onset of increases in subjective ratings of "High" occurred just after smoking began (by 12.5 minutes, difference = 6.768; 95% CI 4.603 8.933; $p\leq0.001$, Fig. 2A). Increases in ratings of "Feel Drug Effects" occurred on a similar time course to those of "High" (by 12.5 minutes, difference = 7.268; 95% CI 4.813 10.535; $p\leq0.001$, Fig. 2B). Ratings of "Like Drug" also increased with a similar time course as "High" (by 12.5 minutes, difference = 7.911; 95% CI 5.325 10.497; $p\leq0.001$, Fig. 2C). Cigarette type significantly impacted positive subjective ratings of "High" ($F(1,9)=12.474$; $p\leq0.001$) and "Feel Drug" ($F(1,9)=14.418$; $p\leq0.001$), with pairwise comparisons indicating that nicotine cigarettes increased ratings more than placebo cigarettes ("High" mean difference = 1.043, 95% CI = 0.463 1.623, $p\leq0.001$; "Feel Drug" mean difference = 1.339, 95% CI = 0.646 2.032, $p\leq0.001$; "Like Drug" mean difference = 0.436, 95% CI = −0.391 1.262, $p=0.301$). There was no effect of abstinence on positive subjective ratings time courses, however, cigarette type was also found to significantly interact with abstinence state on ratings of "Like Drug" ($F(1,9)=6.573$; $p=0.011$) with pairwise comparisons indicating that during abstinence, nicotine smoking increased ratings more than during nonabstinence (mean difference = 1.514, 95% CI = 0.345 2.683, $p=0.011$). Consistent with previous findings in other laboratories, visual examination of the data shown in Fig. 2 suggest that in general, subjective ratings of smoking effects evolve over time in a similar manner regardless of whether cigarettes contain nicotine, and regardless of whether smokers are abstinent from smoking. The similar time courses observed during placebo and nicotine smoking sessions suggest that some of the reported subjective effects of smoking are due to sensory aspects of smoking, such as the taste and smell of smoke, and smoke related airway sensations and not due to the effects of nicotine.

3.2.1. Impact of cigarette type on positive reinforcement during smoking

In order to better dissociate the effects of nicotine from those of conditioned reinforcers, subsequent analyses used only the normalized subjective ratings collected during the three time points during smoking when smoking associated conditioned reinforcers are present (Fig. 3A C). To the extent that nicotine is the primary difference between the nicotine smoking condition and the placebo smoking condition (conditioned reinforcers are the same in both conditions), a significant effect of cigarette type suggests that nicotine is an important determinant of the subjective rating. When time points during smoking were considered, analyses of the effect of cigarette type showed that compared to placebo cigarettes, nicotine containing cigarettes produced significantly more "High" sensation ($F(1,13)=14.885$, $p\leq0.001$; Fig. 3A), significantly greater drug liking, ($F(1,9)=7.949$, $p=0.005$,

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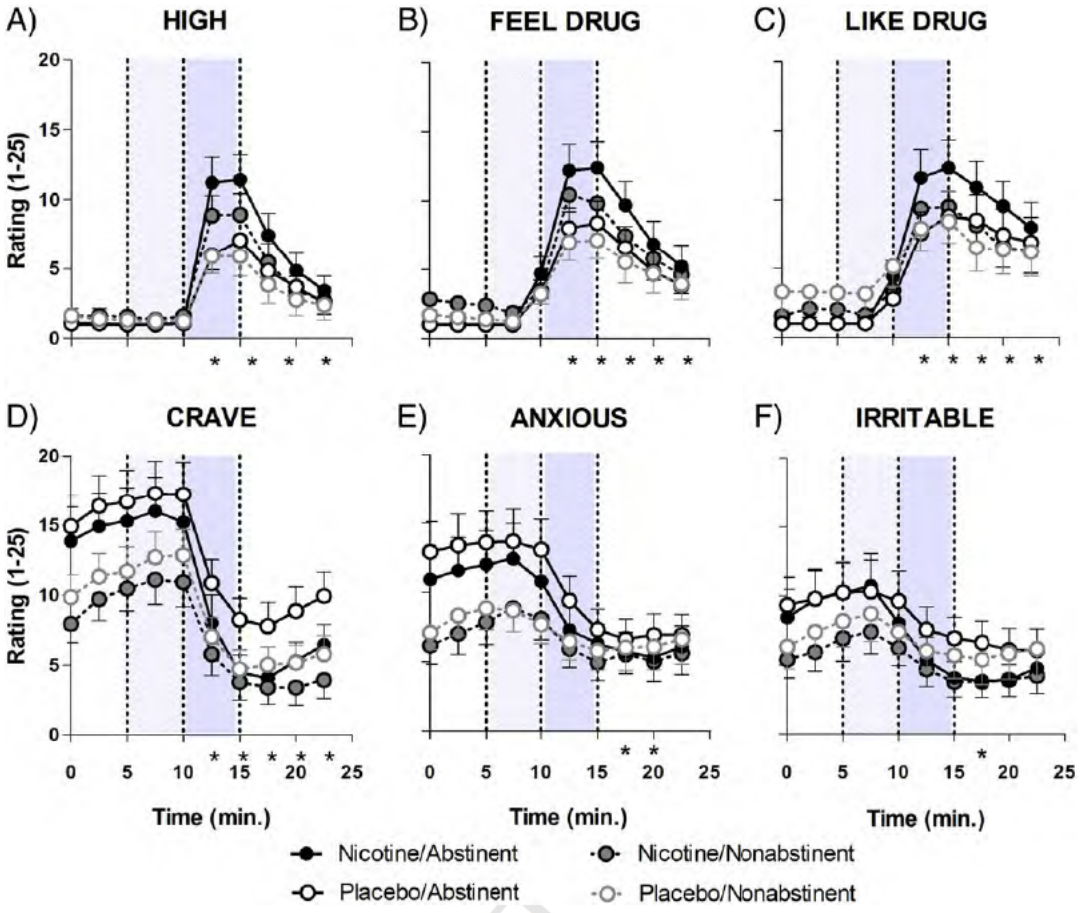


Fig. 2. Full time courses of smoking induced subjective ratings. All four conditions analyzed in the 2×2×9 ANOVA are shown. The air puffing period (minutes 5–10) and smoking period (minutes 10–15) are indicated by light gray and dark gray rectangles respectively. Time points showing a significant main effect of time are indicated on the *X* axis of each panel. (Significance from baseline time-point: $^{*}p \leq 0.05$) Abstinence state exerted a significant main effect on all subjective ratings (Panels A–F). Cigarette type had a significant main effect on "High" (A), "Feel Drug" (B), "Like Drug" (C), and "Crave" (D). Cigarette type and abstinence state interacted significantly on ratings of "Like Drug" (C) with the condition in which a nicotine cigarette was smoked in an abstinent state producing the greatest changes in ratings.

main effect of cigarette type; Fig. 3C), and significantly greater subjective ratings of "Feel Drug Effects" ($F(1,9) = 7.138$, $p = 0.008$; Fig. 3B). These data add to abundant previous evidence that nicotine is an important determinant of subjective ratings of positive reinforcement during smoking. More importantly, the data increase our confidence in the validity of this experimental design as a method to probe additional factors impacting subjective effects of smoking.

3.2.2. Impact of smoking abstinence on positive reinforcement during smoking

The effects of smoking abstinence state were assessed by comparing the ratings of participants' first cigarette of the day to the second. To the extent that the 25 minutes that elapsed between the smoking of the two cigarettes in each session reflects a realistic interval, a significant effect of smoking abstinence state may suggest differences in smoking reinforcement during the daily initiation of smoking compared to during the daily maintenance of smoking. Analyses of the effect of smoking abstinence on subjective ratings data collected during smoking showed that compared to smoking in non abstinence, smoking in abstinence produced significantly greater subjective sensations of "High" ($F(1,9) = 4.044$, $p = 0.046$, Fig. 3A), significantly greater subjective sensations of "Feel Drug Effects" ($F(1,9) = 7.531$, $p = 0.007$, Fig. 3B), and significantly greater ratings of drug liking during smoking ($F(1,9) = 8.181$, $p = 0.007$, Fig. 3C). These data indicate that abstinence is an important determinant of subjective ratings of positive reinforcement during smoking. No interactions between cigarette type and abstinence state were found on ratings of positive reinforcement during smoking in this relatively small sample.

3.3. Impact of cigarette type and abstinence state on the time courses of ratings of negative reinforcement from smoking

Three items on the subjective ratings questionnaire investigated subjective ratings of negatively reinforcing aspects of cigarette smoking, and produced ratings that were likely to decrease with smoking. Analysis of the entire time courses of subjective ratings revealed that subjective ratings of "Crave" changed significantly over time ($F(1,9) = 18.626$, $p \leq 0.001$, Fig. 2D). Ratings of "Crave" decreased significantly from baseline by the end of the cigarette smoking period under every condition (by 15 minutes difference = −6.161; 95% CI 10.288 2.034; $p \leq 0.001$, Fig. 2D). Time was also found to have a significant main effect on ratings of "Anxious" ($F(1,9) = 4.467$, $p \leq 0.001$, Fig. 2E) and "Irritable" ($F(1,9) = 3.105$, $p = 0.001$, Fig. 2F). In analyses of the entire time course, cigarette type significantly influenced ratings of "Crave" ($F(1,9) = 11.870$, $p \leq 0.001$), but did not influence "Anxious" or "Irritable", the other subjective ratings of negative reinforcement. Examination of the craving time courses shows that nicotine smoking produced larger and more persistent reductions in craving than placebo smoking (Fig. 2D). Abstinence state significantly impacted all three ratings of negative reinforcement ("Crave" $F(1,9) = 4.467$, $p \leq 0.001$; "Anxious" $F(1,9) = 16.032$, $p \leq 0.001$; "Irritable" $F(1,9) = 3.105$, $p = 0.001$). There was no significant interaction between cigarette type and

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Please cite this article as: Lindsey KP, et al, Nicotine content and abstinence state have different effects on subjective ratings of positive versus negative reinforcement from smoking, Pharmacol Biochem Behav (2012), http://dx.doi.org/10.1016/j.pbb.2012.11.012

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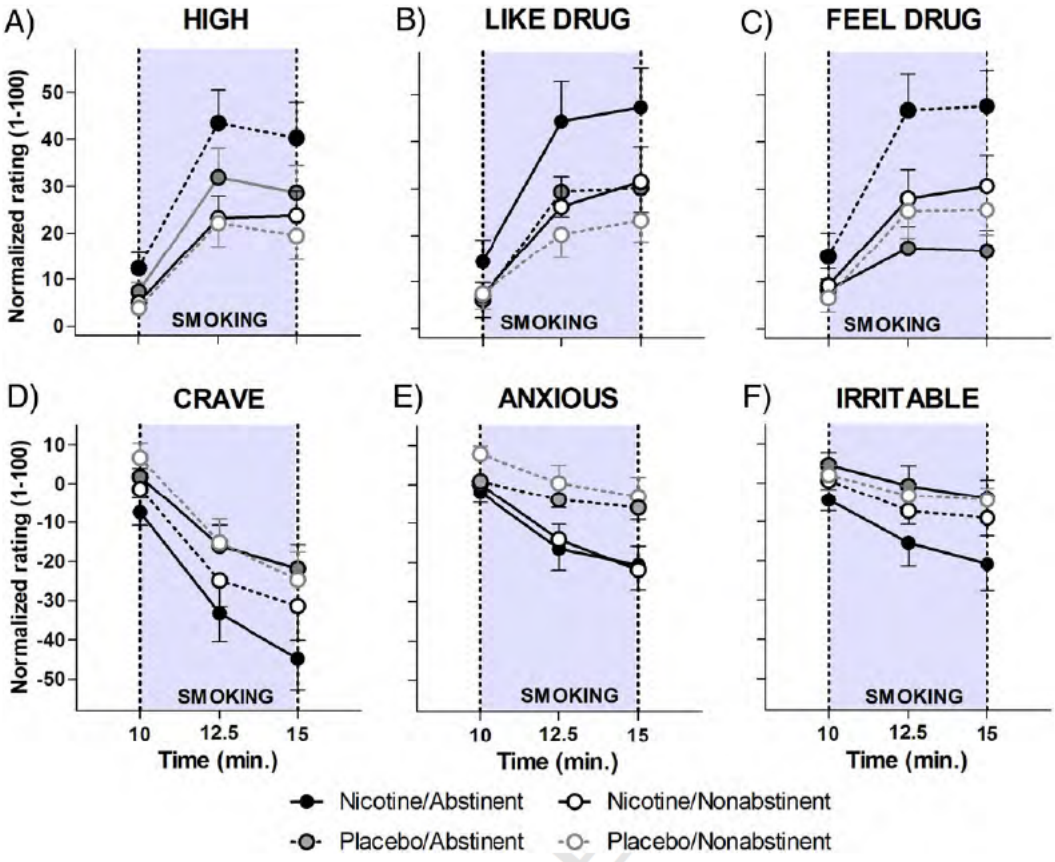


Fig. 3. Subjective ratings in the presence of conditioned reinforcers. All four conditions analyzed in the 2×2×3 ANOVA are shown. The smoking period (minutes 10–15) is indicated in gray. Time points 12.5 and 15 showed a significant main effect of time in all conditions compared to the 10-minute time point at the beginning of smoking (*$p \leq 0.05$). In this analysis, focused on time points when conditioned reinforcers were present, smoking abstinence state exerted a significant main effect on all subjective ratings (A–F). Cigarette type was found to have a significant main effect only on subjective ratings of "High" (A), "Like Drug" (B), and "Feel Drug" (C).

abstinence state on any ratings of negative reinforcement in this rel atively small sample.

3.3.1. Impact of cigarette type on negative reinforcement during smoking

As described in 3.2.1, subsequent analyses of normalized ratings of negative reinforcement considered only the time points in which conditioned reinforcers were present (during which the participant was smoking the cigarette). For these points, analyses of the effects of cigarette type showed that craving was reduced to the same degree regardless of cigarette type ($F(1,9)=2.207$, $p=0.139$, Fig. 3D). Cigarette type also had no effect on smoking induced changes in subjec tive ratings of anxiety ($F(1,9)=0.593$, $p=0.442$, Fig. 3E), or irritability ($F(1,9)=1.853$, $p=0.175$, Fig. 3F). Although caution is warranted when interpreting the absence of significance, the absence of a signifi cant effect of cigarette type on a subjective rating is consistent with the idea that conditioned reinforcers are more important determinants of that rating than is the presence of nicotine. These results are con sistent with the idea that nicotine is important for producing positive reinforcement during smoking, whereas nicotine is not important for producing negative reinforcement during smoking. The results are also consistent with a previous report that nicotine content of cigarettes is not important for relief of induced negative effect in smokers (Perkins et al., 2010). Conversely, conditioned reinforcers appear to play a rela tively larger role in negative reinforcement during smoking.

3.3.2. Impact of smoking abstinence on negative reinforcement during smoking

Analyses of the effect of smoking abstinence on data collected during smoking showed that craving was reduced to a significantly greater degree when a cigarette was smoked during abstinence com pared to non abstinence ($F(1,9)=12.973$, $p \leq 0.001$, Fig. 3D) irrespec tive of cigarette type. As expected, craving was only elevated in the smoking abstinent condition. Additionally, subjective ratings of anxiety and irritability were decreased significantly more by cigarettes smoked in abstinence than cigarettes smoked in non abstinence ("Anxious" $F(1,9)=25.544$, $p \leq 0.001$, Fig. 3E; "Irritable" $F(1,9)=12.292$, $p \leq 0.001$, Fig. 3F). Unlike the effect of cigarette type, the impact of abstinence depended on neither the subjective rating, nor the reinforcer valence; abstinence exerted a significant effect on all subjective ratings of smoking reinforcement whether positive or negative. A significant inter action between cigarette type and abstinence state was found for sub jective ratings of "Irritable", $F(1,9)=4.512$, $p=0.035$, with the largest changes in irritability occurring in the nicotine/smoking abstinent condition (nicotine v. placebo mean difference in ratings of irritability in the abstinent condition = 8.383, $p=0.015$; in the nonabstinent con dition = 1.835, $p=0.59$).

4. Conclusions

Both the presence of nicotine and the state of abstinence from smoking are important determinants of subjective ratings of the effects of smoking. Full time course analyses showed significant main effects of cigarette type on ratings of "High", "Feel Drug" and "Crave". When data were normalized to baseline and only the time points during which conditioned reinforcers were present were con sidered, nicotine content had a significant main effect on all three rat ings of positive reinforcement and no main effect on any of the three ratings of negative reinforcement. These data suggest that nicotine,

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while a critical factor impacting ratings of "High", "Feel Drug" and "Like Drug", may be less relevant to smoking induced changes in craving, anxiety, and irritability. In addition, they add to the growing body of evidence suggesting that the taste, smell, and tracheobroncheal sensations of smoking are at least as important as nicotine self administration for the reduction of craving. The findings support national (United States. Committee on Energy and Commerce, 2009) and international efforts (Scientific Committee on Emerging and Newly Identified Health Risks, 2010); (WHO Study Group on Tobacco Product Regulation, 2012) to regulate all aspects of tobacco products that contribute to the potential for initiation, use, and dependence, including non nicotine factors that may contribute to addictiveness.

Full time course analyses revealed that there were significant main effects of abstinence state on all ratings of negative reinforce ment: "Crave", "Anxiety", and "Irritability". In normalized analyses of time points during which conditioned reinforcers were present, ab stinence state exerted significant effects on both positive and nega tive reinforcement from smoking, with the first cigarette producing larger changes in ratings than the second. These subjective ratings data are intriguingly congruent with increased nicotinic signaling mediated by an upregulated population of nAChRs in abstinence and/or acute desensitization of nAChRs in response to the first ciga rette of the day.

Taken together, these data provide evidence that the presence of nicotine has a greater impact on positively reinforcing effects of ciga rette smoking compared to negatively reinforcing effects. Placebo cigarettes were equally efficacious in producing changes in negatively reinforcing ratings of smoking effects, suggesting that negatively reinforcing effects of cigarette smoking may be influenced to a larger degree by smoking associated conditioned reinforcers than they are by nicotine. Comparisons between the first cigarette of the day and the second suggest that both positive and negative reinforcement from smoking were significantly impacted by smoking abstinence state, consistent with literature reports and current thinking about mechanisms of nicotinic receptor regulation, possibly indicating acute and/or long term tolerance. Both conditioning effects and molecular neuroadaptive mechanisms initiated by both chronic and acute nicotine exposure produce alterations in smoking effects that are reportable by smokers and detectable by investigators armed with relatively simple tools such as subjective ratings questionnaires. Future analyses will attempt to link these changes in subjective ratings to neuroimaging measures of simultaneous regional hemody namic changes in brain in order to better understand the neuronal substrates mediating the subjective effects of nicotine and placebo smoking.

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Please cite this article as: Lindsey KP, et al, Nicotine content and abstinence state have different effects on subjective ratings of positive versus negative reinforcement from smoking, Pharmacol Biochem Behav (2012), http://dx.doi.org/10.1016/j.pbb.2012.11.012

Psychopharmacology (2013) 228:479 486
DOI 10.1007/s00213 013 3054 4

ORIGINAL INVESTIGATION

Reinforcement enhancing effects of nicotine via smoking

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Received: 27 December 2012 / Accepted: 25 February 2013 / Published online: 15 March 2013
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Abstract
Rationale In animals, nicotine enhances reinforcement from stimuli unrelated to nicotine intake. Human research is suggestive but has not clearly shown a similar influence of nicotine.
Objectives We assessed acute effects of nicotine via smoking on enhancement of positive (money, music) or negative (termination of noise) reinforcers, or no "reward" (control). These different rewards determined the generalizability of nicotine effects.
Materials and methods Dependent (n=25) and nondependent (n=27) smokers participated in three sessions, each after overnight abstinence. Using a within-subjects design, sessions involved no smoking or smoking denicotinized (0.05 mg) or nicotine (0.6 mg) Quest^R brand cigarettes. For comparison, a fourth session involved no abstinence prior to smoking one's own brand to gauge responses under typical nicotine satiation. Reinforcement was assessed by responses on a simple operant computer task for the rewards, each available singly on a progressive ratio schedule during separate trials.
Results The reinforcing effect of music, but not other rewards, was greater due to the nicotine cigarette, compared to the denicotinized cigarette or no smoking. Reinforcement enhancing effects of nicotine did not differ between dependent and nondependent groups, indicating no influence of withdrawal relief. Responding due to acute nicotine after abstinence was very similar to responding to one's own brand after no abstinence.
Conclusions Acute nicotine intake per se from smoking after abstinence enhances the reinforcing value of rewards unassociated with smoking, perhaps in a manner comparable to ad lib smoking after no abstinence. Nicotine's reinforcement enhancing effects may be specific to certain rewards, perhaps those sensory in nature.

Keywords Nicotine · Reinforcement enhancement · Reward · Smoking · Dependence

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Introduction

Nicotine is clearly a primary reinforcer, as self-administration behavior of humans and nonhuman animals is increased by contingent nicotine per se (e.g., Harvey et al. 2004; Kenny and Markou 2006; LeFoll and Goldberg 2006). Nicotine intake also can be influenced by secondary reinforcement, in that stimuli directly associated with nicotine can increase responding (i.e., "cues"; Caggiula et al. 2001; Perkins et al. 1994; Rose et al. 2010). Research with animal models (Caggiula et al. 2009; Chaudhri et al. 2006; Olausson et al. 2004; Palmatier et al. 2012; Raiff and Dallery 2008) indicates that nicotine has a third reinforcing function, that of enhancing reinforcement from rewards not directly associated with nicotine intake. This action of nicotine may reflect activation of the mesolimbic dopamine system and perhaps other brain reward pathways (Paterson 2009; Wise 1998). Yet, nicotine's reinforcement enhancing effects may be specific to certain types of rewards and not to any available reinforcing stimulus. For example, some animal studies suggest nicotine may enhance rewards that are "sensory" in nature, such as visual stimuli, but perhaps not most primary rewards, such as food, although this selectivity may also stem from other differences (see Caggiula et al. 2009; Raiff and Dallery 2008). Other stimulant drugs may similarly enhance reinforcement from nondrug rewards (e.g., Sheppard et al. 2012), including sensory types (Lloyd et al. 2012).

In contrast to this animal work, very few studies have directly assessed reinforcement enhancing effects of nicotine in humans. This lack of study is curious since nicotine's ability to enhance reinforcement from stimuli in the environment could help explain the persistence of nicotine intake in humans via tobacco smoking and perhaps the decline in responding for reward after tobacco abstinence (e.g., Powell et al. 2002). Even enhancement of selective rewards may be important; because sensory rewards are common in the natural environment (Fowler 1971; Raiff et al. 2012), including while smoking (e.g., Van Gucht et al. 2010), nicotine enhancement of immediate sensory reinforcement could be a common experience in smokers. Consistent with the idea that nicotine enhances reinforcement from sensory (including visual) rewards, nicotine via smoking may increase subjective attractiveness ratings of viewing faces (Attwood et al. 2009) and self-reported pleasure in response to watching movie clips (Dawkins et al. 2007). On the other hand, very recent research found a modest, but significant, self-reported increase in perceived enjoyment of various "rewarding events" in the first weeks after quitting smoking, although only post-quit perceived change in perception was assessed (Snuggs and Hajek 2013). However, such self-report measures are only indirectly related to reinforcement per se, which generally requires access to a stimulus (i.e., reward) that is contingent on behavioral responding of some kind (e.g., Honig and Staddon 1977).

Other human research has assessed nicotine's influence on responding positively reinforced by money, with somewhat mixed findings. No effect of nicotine via inhaler on gambling behavior for monetary reinforcement was recently reported (McGrath et al. 2012). Nicotine via lozenge increased card-sorting speed for money in heavier smokers (>15 cigs/day), but not in lighter smokers (Dawkins et al. 2006), raising the possibility that these nicotine effects on monetary reward were secondary to dependence, or that they reflected withdrawal relief and not necessarily enhancement of reinforced responding directly. Yet, transdermal nicotine increased response bias on a difficult signal detection task reinforced by money in non- or ex-smokers (Barr et al. 2008), ruling out withdrawal relief. However, because nicotine marginally improved cognitive processing task ability, this responding by nicotine may have been independent of enhancement of reinforcement per se (e.g., Evans and Drobes 2009).

The current study assessed effects of nicotine vs. denicotinized cigarette smoking and vs. no smoking, all after overnight abstinence, on reinforced responding during a simple operant computer task for various rewards in nondependent and dependent smokers. Comparing nicotine vs. denicotinized cigarettes determined nicotine effects per se, and comparing denicotinized cigarettes vs. no smoking determined nonspecific effects of any smoking behavior on reinforced responding. We hypothesized that nicotine per se would increase reinforced responding for one or more of the available rewards, compared to no reward (control for nonspecific responding), in both nondependent and dependent smokers. To gauge generalizability of nicotine effects across types of reward, we compared responding for each of two positive reinforcers, preferred music (e.g., a sensory reward) and money, as well as for the negative reinforcing effects of terminating an aversive loud noise, in a mostly within-subjects design.

We previously reported (Perkins et al. 2009) two studies examining the influence of very low doses of nicotine (via nasal spray or cigarettes) on responding for these same rewards, and no increase in reinforced responding for any rewards was observed with nicotine administration. However, we studied only nondependent, very occasional smokers (<5 cigarettes/week) to completely eliminate any potential effects of withdrawal relief (since nondependent do not experience withdrawal; Shiffman 1989). Consequently, the amount of nicotine intake was limited (e.g., four puffs/trial) in these intermittent smokers to avoid aversive responses, although this intake was sufficient to cause increased subjective responses (e.g., "liking"). Moreover, some procedures may have made the reinforcement task insensitive for assessing maximal responding for reward (breakpoint) within each 15-min trial, namely a 1-sec response delay and a very gradual progressive ratio (PR10%) schedule. Thus, in the current study, we included dependent, as well as nondependent, smokers, provided six puffs/trial, eliminated the response delay, and used a PR30% schedule of reinforcement. Also, for comparison, we added a fourth session involving ad lib smoking of one's preferred brand after no overnight abstinence, to examine the degree to which acute smoking of a nicotine cigarette after abstinence would match reinforced responding under typical non-abstinent smoking conditions.

Method

Participants

Study participants (N=52) comprised of 25 dependent (11 men, 14 women) and 27 nondependent (11 men, 16 women) smokers. Dependent smokers were those who smoked at least 10 cigarettes per day for at least one year and met DSM-IV criteria for nicotine dependence (APA 1994), according to a structured interview updated from Breslau et al. (1994). Nondependent smokers were those who reported never smoking more than five cigarettes per day and who did not ever meet DSM-IV nicotine dependence criteria. Dependent and nondependent smokers differed on some smoking characteristics, as expected, with respective

means (±SD) of 14.3±3.8 vs. 1.5±1.1 cigarettes/day and 4.6±1.9 vs. 0.2±0.6 on the Fagerstrom Test of Nicotine Dependence (Heatherton et al. 1991). The respective nicotine yield of their preferred brand was 1.0±0.2 vs. 0.9± 0.1 mg, 56 vs. 74 % were non-menthol smokers, and they were 29.2±11.4 vs. 21.3±3.2 years of age. Men and women did not differ on these smoking characteristics or age. Participants were recruited from the surrounding community and most self-identified as Caucasian (82.7 %), with 9.6 % African-American, and 7.7 % Asian. We excluded those currently taking medications to treat serious psychological problems (e.g., psychosis, major depression).

CO, withdrawal

Expired air carbon monoxide (CO) was assessed upon arrival via Breath CO monitor (Lenexa, KS) to confirm overnight (>12 h) smoking abstinence (CO <10 ppm; SRNT Subcommittee 2002) in all subjects on three sessions and to confirm nonabstinence (i.e., satiation; CO >10 ppm) in dependent smokers on the comparison ad lib smoking session. CO was also assessed at the end of each session to gauge exposure from session smoking intake (although puff volume prior to each trial was the primary measure; see below). Nicotine withdrawal was assessed by the Minnesota Nicotine Withdrawal Scale (Hughes and Hatsukami 2007) using the following six items: depressed mood/sad, irritable/angry/frustrated, anxious/nervous, difficulty concentrating, restless/impatient, and drowsiness. Items were each rated on a 0 ("not at all") to 100 ("extremely") visual analog scale (VAS) and averaged across symptoms to get a total withdrawal score.

Cigarettes

Subjects smoked on three sessions, involving smoking a moderate nicotine brand, a denicotinized brand, and, for comparison, their own preferred brand. The moderate nicotine brand was Quest^{R} 1 (yield of 0.6 mg nicotine, 9 mg tar), and the denicotinized brand was Quest^{R} 3 (yield <0.05 mg nicotine, 9 mg tar), both formerly sold commercially by Vector Group, Ltd. (Miami, FL). Menthol smokers received menthol Quest; non-menthol smokers received non-menthol Quest. All Quest cigarettes had identifiable markings covered, and subjects were kept blind to brand, but markings of their preferred brand were not covered in the non-abstinent comparison session and so were administered unblind. We included the own brand/satiation condition to compare the reinforcement enhancing effects of nicotine between the acute intake from Quest 1 after overnight abstinence and ad lib smoking intake after no abstinence (typical of most smoking in the natural environment).

Reinforcement task

Reinforced responding was assessed with a modified version of a simple computer task ("Applepicker"; Norman and Jongerius 1985) used in many prior studies by us and others (e.g., Epstein et al.1991; Perkins et al. 1994, 1997, 2009). The task involves using arrow keys to move a cursor on a monitor around a "field" and pressing a button (a "pick") when the cursor lands on one of the "trees" to look for "apples". (The graphical representations are kept rudimentary to minimize any visual entertainment value from responding on the task.) When an "apple" is found, a symbol briefly lights up as feedback, signaling that a unit of the reward designated for that trial (i.e., one reinforcer) has been earned. The number of responses, or "picks," required to find an "apple" constitute the reinforcement schedule, which in this study was a progressive ratio, incrementing by 30 % after each completed ratio (i.e., PR30%), starting with an FR10.

As in prior research (Perkins et al. 2009), each of the four trials per session differed in the reward that was available as a reinforcer over the 15-min of task responding: (1) money (counter incrementing by $.10); (2) preferred music (playing of 30 sec of preferred music from participant's compact disc through headphones; see Procedures below), (3) termination for 30 sec of aversive intermittent noise (four bursts per 30 sec of loud [106 dB] acoustic stimuli through headphones; see Perkins et al. 2009), and (4) no reward (control). The reward available for the current trial was displayed on the monitor once the trial began, and these reward trials were presented in counterbalanced order between subjects. The total money earned was displayed on the counter and added to the total amount for participating. The experimenter was not in the room with participants during task trials; the playing of the music reward was initiated by the computer and began immediately after a reinforcer was earned. Similarly, the intermittent noise was discontinued for 30 sec by the computer immediately after a reinforcer was earned, resulting in no audio through the headphones. Upon earning a reinforcer, subjects could continue responding on the task without interruption, to earn additional units of the reinforcer (i.e., further increment money counter, extend the time of music or termination of aversive noise). They were free to stop responding at any point and simply wait quietly, or read available magazines provided (intentionally routine in nature), until the end of the 15-min task period. (To eliminate any other alternative reinforcers, they were not allowed to bring their own material to sessions.) Virtually all did so on each trial, as mean (SE) duration of responding per trial was 8.7±0.7 min (11.0±0.6 min for money, 10.0±0.5 min for music, 8.7 ±0.6 min to terminate noise, and 5.2±0.7 min for no

reward), indicating that maximal responding for reward was reached (i.e., breakpoint).

Procedure

Participants were first screened by phone on smoking and health history and scheduled for an introductory session in the laboratory to obtain written informed consent and verify eligibility. They also brought with them a compact disc of their own preferred music for use as the music reward in experimental sessions. Participants were instructed to select their five most highly preferred "tracks", and four tracks rated at least 75 on a 0–100 VAS of "liking" were selected as the music reinforcer, totaling more than 10 min of music. They were then introduced to the Applepicker task without reinforcement to become familiar with it during subsequent experimental sessions.

All engaged in four 2-h experimental sessions, with three following overnight abstinence and differing only in the acute smoking condition in effect: nicotine (Quest 1) or denicotinized (Quest 3, "denic") cigarettes presented under blind conditions, or no smoking. A fourth session involved no overnight abstinence and smoking of one's preferred brand (unblinded), provided by the experimenter (to equate the "free" access to all available cigarettes). The order of these smoking conditions across the four sessions was counterbalanced between subjects and separated by at least 1 day. Upon arrival to each session, participants completed the withdrawal and CO measures. Then, prior to each of the four task trials on the three smoking sessions, participants self-administered six puffs over 3 min, one puff every 30 sec, on the cigarette assigned for that session. All smoking was done via the portable Clinical Research Support System ("CReSS Pocket"; Borgwaldt KC, Inc., Richmond VA; www.plowshare.com), which assesses puff volume (in ml; Lee et al. 2003; Perkins et al. 2012). Exact puff timing and duration were guided by computer-presented puffing instructions to standardize intake at about 60 ml per puff (consistent with ad lib puffing; e.g., Perkins et al. 2012), as in our prior studies of controlled smoke exposure, including with the CReSS (Perkins et al. 2010). Also, CO was again obtained after the last trial of each session to gauge exposure during this intermittent smoking from the nicotine and denicotinized cigarettes.

During each trial, participants were instructed that they were free to work on the task for as long as they wanted the designated reward (money, preferred music, termination of aversive noise, no reward). The 24 different orders of rewards across trials were counterbalanced between subjects but remained constant across sessions within subjects. Trials lasted 25 min each, to provide time for controlled intake of six puffs, completion of the 15-min task period, and 5-min brief rest until the next trial. This study protocol was approved by the University of Pittsburgh Institutional Review Board.

Data analyses

All analyses were conducted using IBM SPSS 20.0. The primary dependent measure was the number of task responses on the PR schedule for the various available rewards (money, music, termination of aversive noise, no reward). Preliminary analyses showed no effects of cigarette condition order across sessions, and so data for the three sessions involving overnight abstinence were collapsed across orders in subsequent analyses. In the analyses of variance (ANOVAs) of these responses, the between-subjects factor was dependence level (dependent vs. nondependent), and the within-subjects factors were smoking condition (no smoke, denicotinized cigarette, nicotine cigarette) and type of reward (4). Because no differences in reinforced responding due to dependence were significant (as noted below), most analyses were completely within-subjects, which greatly increased statistical power (e.g., Cohen 1988). Significant effects of smoking condition in all analyses were followed up with paired comparisons of responses (Huitema 1980). Similarly, we also assessed differences in duration of reinforced responding during the 15-min trial, due to reward and cigarette condition. Effect sizes for reinforced responding of particular interest are presented by partial eta-squared values (η_p^2), which indicate the percent of variance explained.

We also conducted similar analyses of responses between the nicotine cigarette session (Quest 1) versus the ad lib/own brand session (i.e., no abstinence), to explore whether acute nicotine intake after overnight abstinence differed from the non-abstinent, satiated condition. Finally, we assessed differences between the ad lib smoking and no smoking conditions to determine effects of complete smoking abstinence on reinforced responding.

Results

Withdrawal and dependence level

Mean (SE) withdrawal upon arrival was significantly increased by overnight abstinence, versus the no abstinence comparison session, for the dependent smokers, 28.6±3.3 vs. 16.9±2.6, respectively, but not for the nondependent smokers, 14.9±3.2 vs. 15.9±2.5. Thus, the interaction of abstinence × dependence level on withdrawal severity was significant, $F(1,50)=9.30$, $p<0.005$, and nondependent smokers showed no withdrawal due to this brief abstinence, as expected.

Control over smoke intake

Mean (SE) puff volume from the six puffs on each trial was greater on the denicotinized vs. nicotine cigarette smoking sessions, 339±13 vs. 265±12 ml, respectively, $F(1,50)=40.07$, $p<0.001$. Yet, there was no main effect of reward (range of 298 ml for money to 308 ml for no reward) and no interaction of nicotine/denicotinized cigarette × reward on puff volume, both $F(3,150)<1$, ns. There were also no significant effects of dependence, $F(1,49)=2.65$, $p=0.11$, cigarette × dependence, $F(1,49)=2.18$, $p=0.15$, or of the interactions of reward × dependence or of reward × cigarette × dependence, both $F(3,147)<1$, ns. Moreover, there was no effect of cigarette × dependence level on CO increase (assessed only at baseline and after the last trial; +14.9± 0.8 ppm), $F(1,50)<1$, ns. In sum, although smoke intake was greater for the denicotinized cigarette, consumption did not differ by reward type or dependence level.

Reinforcing value of rewards

In the three sessions following overnight abstinence, overall responding for rewards was significantly influenced by the main effects of smoking condition, $F(2,100)=10.03$, $p<0.001$, $\eta_p^2=0.167$, reward, $F(3,150)=48.93$, $p<0.001$, $\eta_p^2=0.495$, and the smoking × reward interaction, $F(6,300)=2.13$, $p=0.05$, $\eta_p^2=0.041$. For the main effects of smoking condition, pairwise comparisons (i.e., within-subjects) showed greater mean (SE) reinforced responding per trial due to the nicotine cigarette, 788± 42, which was 116±26 more than the denicotinized cigarette, $F(1,51)=19.90$, $p<0.001$, $\eta_p^2=0.281$, and 111 ±32 more than no smoking, $F(1,51)=11.71$, $p<0.001$, $\eta_p^2=0.187$. In other comparisons, none of these effects varied by dependence/nondependence, as the main effect of dependence level, $F(1,50)<1$, and interactions of dependence × smoking condition, $F(2,100)<1$, dependence × reward, $F(3,150)=1.17$, $p=0.32$, and dependence × smoking × reward, $F(6,300)=1.11$, $p=0.35$, were not significant. Therefore, these effects of smoking on responding for rewards did not differ between the dependent and nondependent subjects, despite their clear difference in withdrawal due to overnight abstinence (above).

Because dependence level had no significant impact on these effects, subsequent analyses examined the source of the smoking × reward interaction on amount of responding in all subjects combined (again using within-subjects comparisons). These follow-up ANOVAs showed significant effects of smoking condition for music reward only, $F(2,102)=10.49$, $p<0.001$, $\eta_p^2=0.171$. Smoking condition only marginally affected responding for money, $F(2,102)=2.72$, $p=0.07$, $\eta_p^2=0.051$, and for termination of aversive noise $F(2,102)=2.37$, $p=0.10$, $\eta_p^2=0.044$, and had no effect on responding for no reward (control), $F(2,102)=1.58$, $p=0.21$. "Earnings" for each respective reward trial averaged 5.9 min of music, $1.20, and 5.2 min of terminating/avoiding intermittent aversive noise.

Mean (SE) responding for each reward is shown in Figure 1 for all subjects by the three smoking conditions after overnight abstinence. Responding was greater for each of the three rewards compared to no reward, confirming they were reinforcers (Honig and Staddon 1977). As in the figure, pairwise comparisons found that reinforced responding for music during the nicotine cigarette increased 181±38 vs. the denicotinized cigarette, $t(51)=4.76$, $p<0.001$, and 241±62 vs. no smoking, $t(51)=3.88$, $p<0.001$. The denicotinized and no smoking conditions did not differ from each other ($t=0.98$, $p=0.33$), indicating

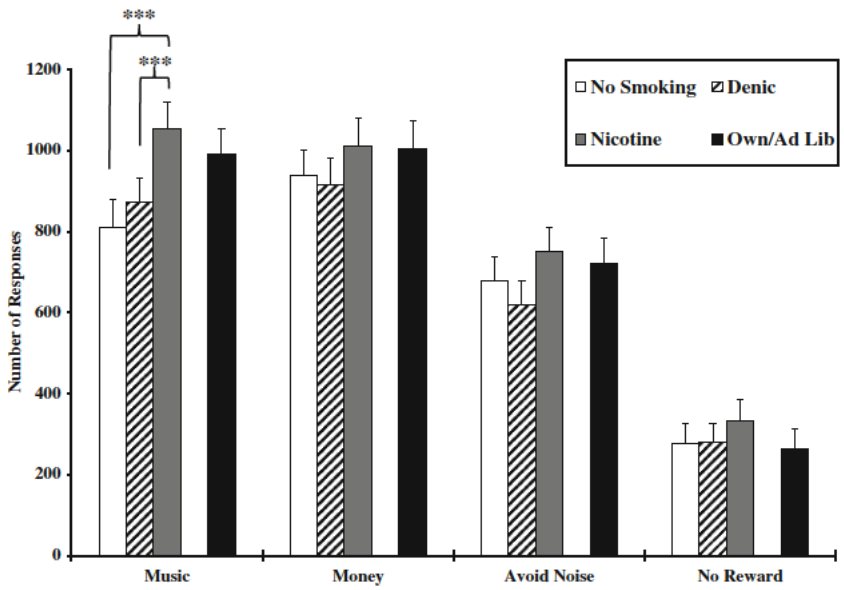


Fig. 1 Mean ± SE total number of responses per trial for reinforcers by reward type and smoking condition ($N=52$). Only music reward was significantly influenced by nicotine intake. *** $p<0.001$ for within subjects paired comparisons of the difference in responding from smoking the nicotine cigarette after overnight abstinence

that nicotine intake per se, and not simply smoking behavior in general, influenced reinforced responding for music reward. Responding for music reward was increased by the nicotine vs. denicotinized cigarette condition in 40 of the 52 subjects (77 %), and music reward responding was correlated 0.80, $p<0.001$, between the nicotine vs. denicotinized cigarette, showing high consistency.

Although the order of rewards across the four trials per session was counterbalanced, we also assessed reinforced responding for music reward by session trial number (i.e., 1–4, in which reward order remained the same for each individual across sessions). We found no effects of trial number, $F(3,48)=1.25$, $p=0.30$, or the interaction of smoking condition × trial number, $F(6, 96)<1$, ns, suggesting that nicotine's enhancement of music reinforcement was similar regardless of whether it was available during earlier or later trials within the session.

Consistent with the amounts of responding, as expected, results were similar for the duration of task responding per trial, with a significant main effect of reward, $F(3,150)=33.77$, $p<0.001$, $\eta_p^2=0.678$, and the smoking × reward interaction, $F(6,300)=2.09$, $p=0.05$, $\eta_p^2=0.252$, but no main effect of smoking condition, $F(2,100)<1$, ns. Overall duration of responding per trial was marginally longer in dependent vs. nondependent smokers, 9.5±0.6 vs. 8.0± 0.6 min, $F(1,50)=2.99$, $p=0.09$, but duration did not vary by the interactions of reward × dependence or smoking × dependence, both F's<1, ns, or of smoking × reward × dependence, $F(6,300)=1.49$, $p=0.18$.

Comparisons with ad lib smoking (no abstinence)

Also shown in Fig. 1 is the reinforced responding for each reward during the ad lib smoking of own brand (no abstinence) comparison condition. Responding for reward was very similar between the ad lib condition and the Quest 1 nicotine cigarette condition after overnight abstinence, $F(1,51)=2.18$, $p=0.15$, $\eta_p^2=0.041$, and there was no significant interaction of ad lib/Quest 1 × reward on reinforced responding, $F(3,153)<1$, ns. So, acute nicotine intake after abstinence enhanced reinforcement as much as ad lib nicotine intake after no abstinence.

However, overall reinforced responding was significantly different for the ad lib smoking vs. no smoking conditions, $F(1,51)=5.69$, $p=0.02$, $\eta_p^2=0.100$, and for the interaction of ad lib smoking/no smoking × reward, $F(3,153)=3.31$, $p=0.02$, $\eta_p^2=0.061$. Again, only reinforcement of music reward was enhanced by ad lib smoking, $F(1,51)=10.18$, $p=0.002$, $\eta_p^2=0.166$, while responding for money, $F(1,51)=1.56$, $p=0.22$, or for termination of aversive noise or for no reward, both $F(1,51)<1$, were not different between ad lib smoking vs. no smoking, further suggesting that nicotine's reinforcement enhancing effects may be specific to certain rewards.

Discussion

To our knowledge, this may be the first clear demonstration in humans that nicotine via smoking has reinforcement enhancing effects, i.e., that it increases reinforcement from stimuli unassociated with nicotine. Because reinforced responses were significantly greater following smoking of nicotine cigarettes, compared to smoking denicotinized cigarettes or no smoking, this reinforcement enhancing effect is due to acute nicotine intake per se and not a general response to smoking behavior. Nicotine did not affect responding for no reward (control trial), ruling out nonspecific effects of nicotine. These reinforcement enhancing effects of nicotine via smoking did not differ between dependent and nondependent smokers, despite their expected differences in withdrawal response to overnight abstinence, indicating these effects are not a consequence of dependence or a result of withdrawal relief. Consequently, animal research results demonstrating nicotine's reinforcement enhancing effects may be very relevant to understanding nicotine effects in humans (Caggiula et al. 2009), suggesting inter-species consistency (e.g., O'Dell and Khroyan 2009).

However, nicotine may enhance reinforcement from only specific types of rewards. With the three types available to smokers in our study, nicotine acted in rather narrow fashion, enhancing the "sensory" reward of music but not the nonsensory reward of money or the negatively reinforcing sensory reward of terminating aversive noise. We found similar overall amounts of responding for the money vs. music rewards, ruling out differential magnitude of reinforcing efficacy as an explanation for this specificity of effects of nicotine on reward type (see Palmatier et al. 2012). Therefore, it is conceivable these nicotine effects may be very specific to certain types of rewards and not broadly enhancing of reinforcement from all types of rewards unassociated with nicotine, such as positive reinforcers not sensory in nature (e.g., money) or negative reinforcers, even if also immediately sensory in nature (terminating aversive noise). Yet, these rewards varied in ways other than their sensory characteristics, such as in immediacy vs. delayed (money) delivery, and in terms of their secondary (money?) versus primary reinforcing actions. Clearly, further research in humans is needed with wider availability of other types of sensory and nonsensory rewards to replicate the specificity of our reinforcement enhancing effects of nicotine and assess whether nicotine may enhance reinforcement from other types of rewards. If our results are confirmed, they may appear consistent with findings from some animal research that nicotine enhances reinforcement from certain rewarding stimuli but not others (e.g., Caggiula et al. 2009; Raiff and Dallery 2008).

Although speculative, our results on the reinforcement enhancing effects of nicotine may have clinical implications, even if the effects are specific to certain types of rewards. For example, even if limited to sensory rewards, nicotine via smoking could increase the degree of enjoyment from many rewarding activities involving "sensory" stimuli, such as watching television, listening to music (as in our task), viewing movies or certain internet websites, or other comparable activities (e.g., Hatsukami et al. 1990; Raiff et al. 2012; Van Gucht et al. 2010). Moreover, loss of this reinforcement could help account for why quitting smoking may produce greater subjective deprivation than expected based solely on loss of direct reinforcing effects of nicotine per se (Baker et al. 2004). Thus, quitting smoking would not only remove primary and secondary reinforcing effects of nicotine (typically the focus of cessation treatments), but also these reinforcement enhancing effects of nicotine, which could decrease overall reinforcement from many daily rewards in a smoker's environment. This notion is consistent with our results, as reinforced responding for music reward was significantly less during the no smoking condition after overnight abstinence, compared to responding during ad lib smoking of own brand after no abstinence, although our participants were not interested in quitting. It is also similar to our recent study in a small sample of smokers high in quit interest, in which responding for music reward was greater during an initial ad lib smoking session than during a subsequent 24-h abstinence session on oral placebo (Perkins et al. 2013). Consequently, a smoking lapse could abruptly increase reinforcement from rewarding activities. This was perhaps illustrated in our study by the fact that acute smoking of the Quest 1 following overnight abstinence was modest in nicotine intake but produced responding for reward similar to that during ad lib smoking of own brand after no abstinence. This finding potentially helps explain why smoking lapses may be particularly reinforcing after a period of cessation and how a single lapse can prompt full blown relapse (Shadel et al. 2011).

As noted, future research should examine the breadth of reinforcement enhancing effects of nicotine across the many types of rewards commonly available to humans when smoking, as well as via different methods of nicotine administration. Such studies may be able to ascertain whether greater reinforced responding stems from an increase in the hedonic value of a reward or from a decrease in the perceived cost of responding (e.g., Salamone 2006). Nicotine's reinforcement enhancing effects also may depend on the nature of alternatives to responding for some rewards, or the specific conditions (e.g., feeling subjective distress may further enhance reinforced responding for certain rewards but attenuate responding for other rewards). Subsequent research also could examine individual differences in the magnitude of reinforced responding due to nicotine and/or the loss of such responding after quitting (e.g., Van Voorhees et al. 2012). Research should continue determining the changes in brain activation due to acute nicotine and relating these to its reinforcement enhancing effects, especially in smokers (Addicott et al. 2012; Guy and Fletcher 2013; Klinkenberg et al. 2013; Paterson 2009; Wise 1998). Finally, effects of other drugs on enhancement of reinforcement in humans warrant study (e.g., Lloyd et al. 2012; Perkins et al. 2013; Sheppard et al. 2012).

Acknowledgment This research was supported by NIH Grant DA31218 (KAP). Neither author has any potential conflicts of interest to report.

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Original Investigation

Separate and Combined Effects of Very Low Nicotine Cigarettes and Nicotine Replacement in Smokers with Schizophrenia and Controls

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Received January 13, 2012; accepted February 26, 2012

Abstract

Introduction: The prevalence of smoking among people with schizophrenia in the United States is about 3 times that of the general population. Novel approaches are needed to reduce rates of smoking-related morbidity and mortality among these smokers.

Methods: This study used a within-subjects design to investigate the separate and combined effects of sensorimotor replacement for smoking (very low nicotine content [VLNC] cigarettes vs. no cigarettes) and transdermal nicotine replacement (42 mg nicotine [NIC] vs. placebo [PLA] patches) in smokers with schizophrenia (SS; $n = 30$) and control smokers without psychiatric illness (CS; $n = 26$). Each session contained a 5-hr controlled administration period in which participants underwent the following conditions, in counterbalanced order: VLNC + NIC, VLNC + PLA, no cigarettes + NIC, no cigarettes + PLA, usual-brand cigarettes + no patches. Next, participants completed measures of cigarette craving, nicotine withdrawal, smoking habit withdrawal, and cigarette subjective effects, followed by a 90-min period of *ad libitum* usual-brand smoking.

Results: Smoking VLNC cigarettes during the controlled administration periods reduced cigarette craving, nicotine withdrawal symptoms, habit withdrawal symptoms, and usual-brand smoking in SS and CS relative to the no cigarette conditions. VLNC cigarettes were well accepted by both groups and did not affect psychiatric symptom levels in SS. Transdermal nicotine significantly reduced cigarette craving but did not affect usual-brand smoking.

Conclusions: These findings suggest that reducing the nicotine content of cigarettes to nonaddictive levels may be a promising approach for reducing nicotine dependence among people with schizophrenia.

Introduction

The prevalence of smoking among people with schizophrenia in the United States is about 60%, which is 3 times that of the general population (de Leon & Diaz, 2005; McClave et al., 2010). Schizophrenia is associated with a 20% reduction in lifespan, due primarily to cardiovascular and other smoking-related illnesses (Brown, Kim, Mitchell, & Inskip, 2010; Hennekens, Hennekens, Hollar, & Casey, 2005; Parks, Svendsen, Singer, Foti, & Mauer, 2006; Saha, Chant, & McGrath, 2007). Although almost 40% of smokers with schizophrenia (SS) in the 2007 National Health Interview Survey reported having tried to quit smoking in the past year (McClave et al., 2010), SS have high relapse rates even when they have access to combined pharmacological and psychosocial smoking treatments (e.g., Evins et al., 2007; George et al., 2002). Thus, there is a need for innovative and effective approaches to improving smoking cessation rates among these smokers.

A recent human laboratory study found that acute use of 42 mg transdermal nicotine replacement reduced nicotine withdrawal symptoms but not usual-brand smoking in SS (Tidey, Rohsenow, Swift, Kaplan, & Adolfo, 2008), suggesting that complementary strategies may be needed to reduce smoking in this population. Very low nicotine content (VLNC) cigarettes (i.e., cigarettes with less than 0.2 mg Federal Trade Commission nicotine yield) provide sensorimotor replacement for usual-brand smoking by imitating the taste, aroma, and respiratory effects of normal nicotine content cigarettes. Among smokers without psychiatric illness, VLNC cigarettes consistently reduce cigarette craving, negative affect, and usual-brand smoking in laboratory and treatment studies (e.g., Buchhalter, Acosta, Evans, Breland, & Eissenberg, 2005; Butschky, Bailey, Henningfield, & Pickworth, 1995; Dallery, Houtsmuller, Pickworth, & Stitzer,

doi:10.1093/ntr/nts098
Advance Access publication April 19, 2012
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2003; Gross, Lee, & Stitzer, 1997; Johnson, Bickel, & Kirshenbaum, 2004; Pickworth, Fant, Nelson, Rohrer, & Henningfield, 1999; Rose, Behm, Westman, & Johnson, 2000; Rose, Behm, Westman, Bates, & Salley, 2003; Westman, Behm, & Rose, 1996). As sensorimotor stimuli that are repeatedly associated with nicotine delivery acquire, through classical conditioning processes, conditioned reinforcing effects that help to maintain smoking (Palmatier et al., 2007; Rose & Levin, 1991), protracted use of VLNC cigarettes in combination with nicotine replacement may improve smoking cessation rates by uncoupling the pharmacological effects of nicotine from these sensorimotor stimuli, thus facilitating the extinction of these conditioned reinforcing effects (e.g., Becker, Rose, & Albino, 2008; Donny & Jones, 2009). Two studies have compared the effects of VLNC versus high nicotine cigarettes on psychiatric symptoms and cognitive performance in SS (Smith, Infante, Ali, & Nigam, 2001; Smith, Singh, Infante, Khandat, & Kloos, 2002). However, neither study investigated the effects of VLNC cigarettes on cigarette craving, nicotine withdrawal, or smoking behavior. To our knowledge, the current study is unique in its focus on the effects of VLNC cigarettes on smoking measures in SS.

The current study investigated the separate and combined effects of acute nicotine replacement and sensorimotor smoking replacement, in the form of VLNC cigarettes, on cigarette craving, withdrawal symptoms, and usual-brand smoking in SS and control smokers (CS). We hypothesized that smoking VLNC cigarettes would reduce cigarette craving, nicotine withdrawal symptoms, smoking habit withdrawal symptoms, and usual-brand cigarette smoking in both SS and CS, and that nicotine replacement would enhance these effects.

Methods

Participants

Participants were required to either have a diagnosis of schizophrenia or schizoaffective disorder (SS) or no Axis I disorder (CS), based on the Structured Clinical Interview for *DSM-IV* (First, Spitzer, Gibbon, & Williams, 1994), to be at least 18-years old, to have smoked 20–50 cigarettes/day for at least the past year, to have a score of at least 6 on the Fagerström Test for Nicotine Dependence (FTND), indicating a high level of dependence (Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991), and to indicate an interest in quitting smoking someday. Exclusionary criteria included medical conditions contraindicating transdermal nicotine use, severe levels of disorientation or uncooperativeness, positive urine drug or pregnancy tests at baseline, or positive breath alcohol level at any session. Procedures were approved by the Institutional Review Board of Brown University. Thirty-seven SS and 38 CS enrolled, and 30 SS and 26 CS completed the study.

Design Overview

A within-subjects design was used to investigate the separate and combined effects of sensorimotor replacement for smoking (VLNC cigarettes vs. no cigarettes) and transdermal nicotine replacement (42 mg nicotine [NIC] vs. placebo [PLA] patches) in SS and CS. In Session 1, participants completed individual difference measures and practiced smoking through the topography measurement device. In Session 2, participants smoked their usual cigarettes *ad libitum* for 5 hr through the topography device so that the rate and timing of their smoking behavior could be determined. In Sessions 3–7, participants underwent the following conditions during 5-hr controlled administration periods, with order counterbalanced across participants: VLNC + NIC, VLNC + PLA, no cigarettes + NIC, no cigarettes + PLA, usual brand cigarettes + no patches. After the 5-hr controlled administration periods, participants completed the subjective measures described below, followed by a 90-min period of *ad libitum* usual-brand smoking. Participants were compensated $500 for completing the study.

Procedure

All sessions began at approximately 9 a.m. Upon arrival for each session, participants provided breath samples for the assessment of expired-air carbon monoxide (CO) levels (Smokerlyzer, Bedfont Scientific Ltd., Kent, U.K.). In Session 1, participants completed the individual difference measures described below and then were asked to smoke 1 cigarette of their usual brand using the smoking topography measurement device (laboratory-based Clinical Research Support System [CReSS], Borgwaldt KC, Richmond, VA) in order to habituate to smoking through the CReSS mouthpiece. The CReSS, which was calibrated before each session, provides the following smoking variables: puffs per cigarette, puff volume, puff duration, maximum flow (peak puff intensity), and inter-puff-interval. In Session 2, participants smoked their usual-brand cigarettes using the CReSS equipment for 5 hr so that characteristics of their typical smoking behavior could be measured.

In Sessions 3–7, participants underwent the following conditions during 5-hr controlled administration periods, with order counterbalanced across participants: VLNC + NIC, VLNC + PLA, no cigarettes + NIC, no cigarettes + PLA, usual brand cigarettes + no patches. PLA or NIC patches (GlaxoSmithKline, Parsippany, NJ) were applied to participants' upper arms (one per arm, for a total of 0 or 42 mg NIC) under double-blind conditions. The VLNC cigarettes used in this study (Quest 3; Vector Tobacco, Timberlake, NC) contained less than 0.05 mg nicotine and 10 mg tar. Usual-brand cigarettes were provided by the experimenters. During the 5-hr controlled administration periods, all cigarettes were smoked through the CReSS, and participants were cued to smoke according to the rate and timing of their smoking during Session 2. Participants were also able to read magazines and watch videos and were provided with a light lunch. Participants were under continuous observation throughout the sessions.

After the 5-hr controlled administration periods, cigarette craving levels, nicotine withdrawal symptoms, smoking habit withdrawal symptoms, cigarette acceptability, and psychiatric symptoms (in SS) were assessed using the measures described below. Assessments of cognitive performance and cigarette demand were also administered but are not described here. Next, breath CO level was measured and participants were instructed that they could smoke as little or as much as they wanted of their usual-brand cigarettes through the CReSS for the next 90 min. At the end of the smoking period, breath CO level was measured and patches were removed.

Measures

Baseline Characteristics

Individual difference measures collected in Session 1 included demographic characteristics, smoking history, and the

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Contemplation Ladder (Biener & Abrams, 1991), a 10-point scale that measures motivation to quit smoking. In SS, current psychiatric symptom levels were measured using the Positive and Negative Syndrome Scale (Kay, Fiszbein, & Opler, 1987). All participants provided a saliva sample for cotinine analysis (Salimetrics LLC, State College, PA).

Sessions 3–7: Behavioral Measures

The primary measure of smoking during the 90-min *ad libitum* usual-brand smoking periods was CO boost (CO level at the end of the 90-min *ad libitum* period minus CO level before the 90-min *ad libitum* period) as this was the most sensitive measure of smoking behavior in a previous study (Tidey et al., 2008). The secondary measure of smoking behavior was total volume of puffs smoked during the 90-min *ad libitum* period as prior studies indicated the sensitivity of this measure to VLNC cigarettes and nicotine replacement in nonpsychiatric smokers (Rose et al., 2003; Strasser, Lerman, Sanborn, Pickworth, & Feldman, 2007).

Sessions 3–7: Subjective Measures

Self-report measures of urge to smoke, nicotine withdrawal symptoms, smoking habit withdrawal symptoms and subjective effects of cigarettes were administered electronically. Urge to smoke was assessed using the Questionnaire on Smoking Urges-brief form (QSU-brief; Cox, Tiffany, & Christen, 2001) and the item "How much is your urge to smoke right now?", which was rated on a 100 mm visual analogue scale with the anchors 0 = *no urge at all* and 100 = *strongest urge you've ever had*. Nicotine withdrawal symptoms were measured using the 8-item Minnesota Nicotine Withdrawal Scale (MNWS), with the insomnia item omitted as participants did not undergo overnight abstinence, and the craving item omitted to allow independent assessment of withdrawal versus craving (Hughes & Hatsukami, 1986, 1998). Each symptom was rated from 0 (*not present*) to 4 (*severe*), and a total symptom score was calculated by averaging scores of each item. Smoking habit withdrawal was measured using two items: "missed something to do with hands" and "missed having something in the mouth," rated from 1 (*not at all*) to 7 (*extremely*; Rose, Behm, Westman, & Johnson, 2000). Subjective effects of cigarettes were measured using the Cigarette Effects Scale (CES; Rose et al., 2000), which consists of 11 items, rated from 0 (*not at all*) to 7 (*extremely*), forming the subscales Satisfaction, Psychological Reward, Nausea/Dizziness, Craving Relief, and Enjoyment of Airway Sensations. Finally, a trained rater assessed psychiatric symptoms in SS using the Brief Psychiatric Rating Scale (BPRS; Overall & Gorham, 1962), an 18-item measure of positive and negative schizophrenia symptoms.

Data Analysis

Group comparisons on demographic and smoking history measures were conducted using independent-samples *t* tests for continuous variables and chi-square tests for categorical variables. CO boosts and total volume of puffs smoked during the 5-hr controlled administration periods were compared using 2 × 3 analyses of variance tests (ANOVAs) with the factors Diagnosis (SS, CS) and Cigarette Condition (VLNC + NIC, VLNC + PLA, usual brand). Comparisons of the effects of nicotine and sensorimotor replacement on QSU-brief, MNWS, Habit Withdrawal, and usual-brand smoking in SS and CS were conducted using mixed-factor 2 × 2 × 2 ANOVAs with the between-groups factor Diagnosis (SS, CS), and the within-subjects factors Nicotine Replacement (NIC, PLA) and Sensorimotor Replacement (VLNC cigarettes, No cigarettes). In addition, 2 × 2 ANOVAs were conducted to compare the NIC + VLNC and usual brand conditions in SS and CS. Comparisons of the subjective effects of VLNC, with and without nicotine replacement, and usual-brand cigarettes were conducted using 2 x 3 ANOVAs with the factors Diagnosis (SS, CS) and Cigarette Condition (VLNC + NIC, VLNC + PLA, usual brand). Effects of nicotine and sensorimotor replacement on BPRS scores in SS were analyzed using 2 × 2 ANOVAs, and *t* tests were conducted to compare BPRS scores from the VLNC + NIC and usual brand conditions. Analyses were conducted using PASW Statistics 17.0 for Windows (SPSS, Inc.). Differences were considered significant when $p \leq .05$. Effect sizes (Cohen's *d*) are also provided when $p = .05$–$.10$ (Cohen, 1988). Significant interactions were followed by simple effects tests. Due to technical errors or malfunctioning equipment, breath CO data were incomplete from two CS, subjective measures were incomplete from four SS and two CS, and puff volume data were incomplete from four SS and eight CS.

Results

Sample Characteristics

The groups did not differ significantly on any demographic or smoking history measure (Table 1). Overall, participants were 45.2 ± 9.5 (*M* ± *SD*) years old, 43% female, 70% White, 19% African American, and had completed 12.1 ± 2.0 years of education. At enrollment, participants smoked 25.1 ± 8.4 cigarettes/day,

Table 1. Baseline Characteristics of Study Participants

	SS ($n = 30$)	CS ($n = 26$)
Age, *M* (*SD*)	46.2 (8.0)	44.5 (10.9)
Male (%)	60	58
Race (%)		
White	77	65
African American	13	23
Hispanic ethnicity	4	0
Employed full- or part-time (%)	7	23
Years of education	11.9 (2.2)	12.2 (1.9)
Cigarettes per day	25.9 (9.9)	23.5 (6.5)
Nicotine dependence (FTND score)	6.9 (1.5)	6.7 (1.8)
Contemplation Ladder Score	5.1 (2.0)	4.7 (1.8)
Years of daily smoking	27.4 (8.3)	28.4 (11.9)
Salivary cotinine level (ng/ml)	454 (267)	434 (240)
Baseline CO level (ppm)	30.6 (20.6)	27.3 (15.2)
PANSS positive scale score	11.6 (4.1)	
PANSS negative scale score	14.6 (6.4)	
PANSS general scale score	25.9 (5.9)	
PANSS total score	52.1 (14.6)	
Antipsychotic drug class	67% Atyp, 17% Typ, 10% both types	

Note. SS = smokers with schizophrenia; CS = control smokers; FTND = Fagerström Test for Nicotine Dependence; CO = expired-air carbon monoxide; PANSS = Positive and Negative Syndrome Scale; Atyp = atypical; Typ = typical.

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had been smoking daily for 28.2 ± 10.0 years, had FTND scores of 6.9 ± 1.7, indicating high levels of nicotine dependence, and had Contemplation Ladder scores of 4.9 ± 1.9, indicating that they were thinking about quitting smoking but did not have immediate plans to quit. SS were clinically stable with low to moderate psychiatric symptom levels, similar to those reported by other studies of smoking in SS (e.g., Fonder et al., 2005; George et al., 2000).

Smoking During the 5-hr Controlled Administration Periods

SS had higher smoke intake levels than CS during the 5-hr controlled administration periods, based on both CO boost ($F(1, 54) = 4.00$, $p = .05$) and total puff volume ($F(1, 44) = 16.39$, $p < .01$). Average CO boosts from the controlled administration periods were 15.7 ± 13.8 ppm in SS and 10.1 ± 11.5 ppm in CS; average total puff volumes from these periods were 7,145 ± 3,995 ml in SS and 3,574 ± 1,641 ml in CS. There was a significant main effect of Cigarette Condition on total puff volume from the controlled administration periods ($F(2, 88) = 3.91$, $p < .05$), with post-hoc comparisons indicating that average total puff volume from the VLNC + NIC condition (4,969 ± 3,594 ml) was significantly lower ($p < .01$) than that from the usual brand condition (5,841 ± 4,004 ml), whereas the total puff volume from the VLNC + PLA condition (5,268 ± 3,309 ml) was intermediate and did not significantly differ from either of those conditions. There was no significant effect of cigarette type on CO boost and no significant interaction between diagnosis and cigarette type on either measure.

Subjective Effects of Sensorimotor and Nicotine Replacement

Figure 1 shows the effects of sensorimotor and nicotine replacement on QSU-brief, MNWS, and Habit Withdrawal scores in SS and CS. SS reported significantly higher MNWS and Habit Withdrawal scores than CS ($F(1, 50) = 22.10$, $p < .001$; $F(1, 50) = 6.84$, $p < .05$; respectively). Averaged across nicotine and sensorimotor replacement conditions, MNWS scores were 34.1 ± 23.1 in SS and 12.8 ± 12.7 in CS, and Habit Withdrawal scores were 3.9 ± 1.5 in SS and 2.9 ± 1.8 in CS. There was no effect of diagnosis on QSU-brief score.

There were significant main effects of sensorimotor replacement on QSU-brief, MNWS, and Habit Withdrawal scores ($F(1, 50) = 84.75$, $p < .001$; $F(1, 50) = 38.77$, $p < .001$; $F(1, 50) = 13.36$, $p < .01$; respectively), with lower scores from the VLNC cigarette conditions compared with the no cigarette conditions. Averaged across diagnostic groups and nicotine replacement conditions, QSU-brief scores were 5.0 ± 1.5 in the no cigarette and 3.2 ± 1.8 in the VLNC cigarette condition, MNWS scores were 29.5 ± 24.1 in the no cigarette and 17.3 ± 20.8 in the VLNC cigarette condition, and Habit Withdrawal scores were 3.7 ± 1.8 in the no cigarette and 3.1 ± 1.6 in the VLNC cigarette condition. There were no significant interactions between diagnosis and sensorimotor replacement on these measures.

There was a significant main effect of nicotine replacement on QSU-brief score ($F(1, 50) = 8.56$, $p < .01$), and the effect of

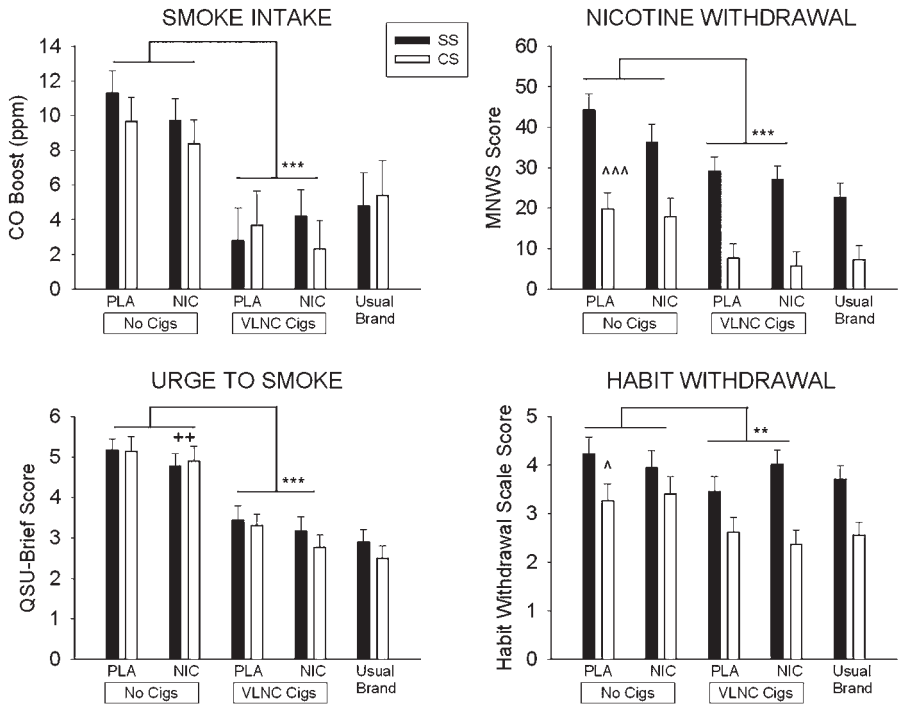


Figure 1. Effects of 5-hr exposure to 42 mg transdermal nicotine (NIC) or placebo patches (PLA) and very low nicotine content cigarettes (VLNC Cigs) or no cigarettes (No Cigs) on CO boost, urge to smoke, nicotine withdrawal symptoms and habit withdrawal symptoms in smokers with schizophrenia (SS; solid bars) and control smokers without psychiatric illness (CS; open bars). Bars represent $M \pm SEM$. Significant main effects of sensorimotor replacement are indicated with asterisks (**$p < .01$; *** $p < .001$), significant main effects of nicotine replacement are indicated with plus signs (++ $p < .01$), and significant main effects of group are indicated with carets (^ $p < .05$; ^^^ $p < .001$).

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nicotine replacement on MNWS score approached significance ($F(1, 50) = 3.21$, $p = .08$; $d = 0.16$). Averaged across diagnostic groups and sensorimotor replacement conditions, QSU-brief scores were 3.9 ± 1.7 in the NIC condition and 4.3 ± 1.6 in the PLA condition, and MNWS scores were 21.7 ± 22.32 in the NIC condition and 25.2 ± 22.52 in the PLA condition. There were no significant Diagnosis × Nicotine Replacement or Sensorimotor Replacement × Nicotine Replacement interactions on these measures. However, there was a significant Diagnosis × Sensorimotor Replacement × Nicotine Replacement interaction effect on Habit Withdrawal score ($F(1, 50) = 5.44$, $p < .05$). Separate 2 × 2 ANOVAs conducted within each diagnostic group indicated that, in SS, there was a significant Nicotine Replacement × Sensorimotor Replacement interaction on Habit Withdrawal Scale score ($F(1, 25) = 5.75$, $p < .05$), and simple effects tests showed that in SS sensorimotor replacement reduced Habit Withdrawal Scale scores within PLA conditions ($t(25) = 3.52$, $p < .01$) but not NIC conditions (Figure 1). In CS, sensorimotor replacement reduced Habit Withdrawal Scale scores ($F(1, 25) = 12.20$, $p < .005$), with no main effect of Nicotine Replacement or interaction between these factors. There were no significant differences between the VLNC + NIC and usual-brand conditions on any of these measures.

Effects of Sensorimotor and Nicotine Replacement on Usual-Brand Smoking

Effects of sensorimotor and nicotine replacement on CO boost from the 90-min *ad libitum* usual-brand smoking periods are shown in Figure 1. There was no effect of diagnosis on CO boost from the *ad libitum* usual-brand smoking periods (SS: 6.98 ± 1.0 ppm; CS: 5.98 ± 1.1 ppm), however, there was a significant effect of diagnosis on total volume smoked during the *ad libitum* usual-brand smoking periods, with higher total puff volume in SS than CS ($F(1, 44) = 8.81$, $p < .01$; SS: 2,536 ± 1,656 ml; CS: 1,451 ± 835 ml; not shown). There were significant main effects of sensorimotor replacement on both CO boost and total volume smoked during the *ad libitum* usual-brand smoking periods ($F(1, 54) = 47.05$, $p < .001$; $F(1, 44) = 12.93$, $p < .01$, respectively). Means indicated that smoking VLNC cigarettes during the controlled administration periods reduced usual-brand smoking during the *ad libitum* periods, based on both CO boost (VLNC cigarette: 3.2 ± 0.2 ppm; No cigarette: 9.8 ± 7.1 ppm) and total puff volume (VLNC cigarette: 1,803 ± 1,420 ml; No cigarette: 2,184 ± 1,498 ml). There was no effect of nicotine replacement or significant interactions among factors on CO boost or total puff volume smoked during the *ad libitum* usual-brand periods.

VLNC Tolerability and Acceptability

Comparisons between VLNC cigarettes with and without nicotine replacement and usual-brand cigarettes on CES scores are shown in Figure 2. There were significant main effects of Diagnosis on the Reward, Nausea/Dizziness, Craving Relief, and Enjoyment of Airway Sensations subscales of the CES, indicating that across cigarette types, SS provided higher ratings on these measures than CS ($F(1, 50) = 7.94$, $p < .01$, $F(1, 50) = 16.04$, $p < .001$; $F(1, 50) = 4.14$, $p < .05$; $F(1, 50) = 10.77$, $p < .01$; respectively). There were significant main effects of cigarette condition on the satisfaction and reward subscales of the CES ($F(2, 100) = 12.63$, $p < .001$; $F(2, 100) = 8.63$, $p < .001$; respectively). Post-hoc tests indicated that, averaged across diagnostic groups, usual-brand cigarettes received higher satisfaction and reward ratings than VLNC + PLA or VLNC + NIC. There was a significant main effect of cigarette condition and a significant Diagnosis × Cigarette Condition interaction on craving relief ($F(2, 100) = 3.34$, $p < .05$; $F(2, 100) = 3.33$, $p < .05$; respectively).

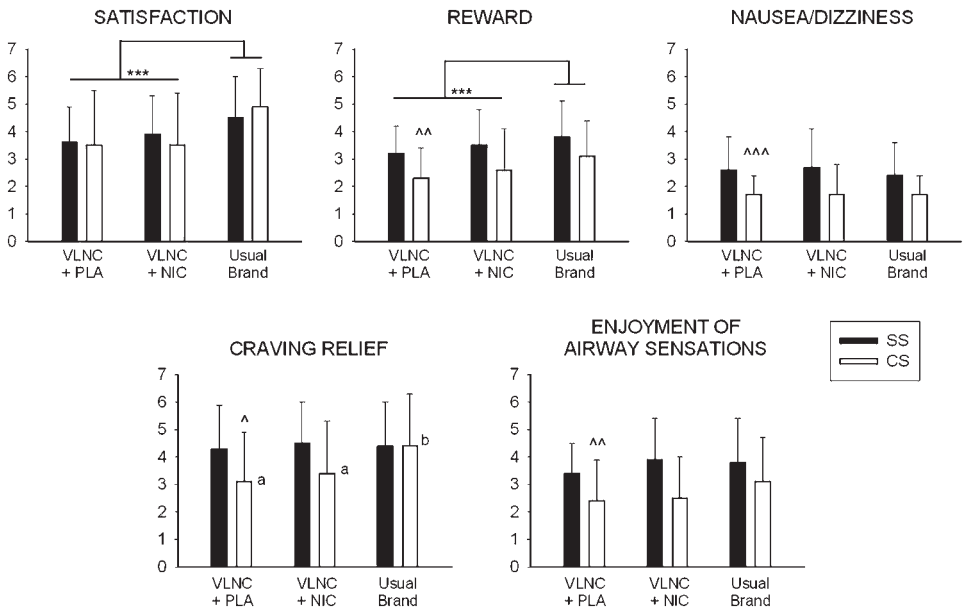


Figure 2. Comparisons of usual-brand cigarettes, very low nicotine content cigarettes plus 42 mg transdermal nicotine (VLNC + NIC) and very low nicotine content cigarettes plus placebo patches (VLNC + PLA) on subscales of the Cigarette Effects Scale in smokers with schizophrenia (SS, solid bars) and control smokers (CS, open bars). Bars represent $M \pm SEM$. Significant main effects of cigarette type are indicated with asterisks (**$p < .01$; *** $p < .001$), significant main effects of group are indicated with carets (^ $p < .05$; ^^ $p < .01$; ^^^ $p < .001$), and different letters (a, b) represent a significant difference within the CS group only.

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Separate ANOVAs and post-hoc tests conducted for each diagnostic group indicated that CS gave higher Craving Relief ratings to usual-brand cigarettes than either VLNC + PLA or VLNC + NIC ($F(2, 50) = 6.74$, $p < .01$), however, SS gave similar Craving Relief ratings under all conditions (Figure 2). BPRS scores in SS were not affected by nicotine or sensorimotor replacement and averaged 24.8 ± 6.2 across conditions (not shown).

Discussion

The results of this study indicate that smoking VLNC cigarettes during the controlled administration periods reduced cigarette craving, nicotine withdrawal symptoms, smoking habit withdrawal symptoms, and usual-brand smoking in both SS and CS. Furthermore, VLNC cigarettes were well tolerated overall, and there was no indication that acute VLNC smoking affected psychiatric symptoms in SS. Transdermal nicotine substituted less effectively than sensorimotor replacement for usual-brand cigarettes under these study conditions, in that it reduced cigarette craving but to a lesser extent than did VLNC cigarettes, and had no effect on usual-brand smoking during the 90-min *ad libitum* smoking periods. These findings are consistent with previous laboratory reports in smokers without psychiatric illness (e.g., Johnson et al., 2004; Rose et al., 2003; Westman et al., 1996). Overall, the results of this study set the stage for longer-term studies of VLNC cigarettes in SS to determine whether reducing the nicotine yield in cigarettes to nonaddictive levels may be an effective strategy for improving smoking cessation outcomes in this population.

A likely explanation for the results of this study are that VLNC cigarettes provide sensorimotor effects that, after years of being paired with nicotine intake while smoking, have acquired conditioned reinforcing effects (Rose & Levin, 1991). Over longer periods of use, the conditioned reinforcing effects of VLNC cigarettes are expected to extinguish, leading to smoking reductions (Rose & Behm, 2004). The potential problem with this approach is that switching from normal nicotine content to VLNC cigarettes could increase carcinogen exposure if smokers compensate for the reduction in nicotine yield by smoking more intensely (reviewed in Scherer, 1999; Rose 2006). Results from the small number of studies that have examined this question indicate that total volume smoked of VLNC cigarettes increases relative to total volume smoked of higher-nicotine research cigarettes under acute smoking conditions (Strasser et al., 2007), but after 4–6 weeks of VLNC cigarette use, smoking rates, carcinogen biomarkers, and nicotine dependence severity decline, suggesting that compensatory smoking of VLNC cigarettes may not persist beyond a period of weeks or months in smokers without serious mental illness (Donny, Houtsmuller, & Stitzer, 2007; Donny & Jones, 2009; Hatsukami, Kotlyar, et al., 2010).

Although these results are promising, several differences between SS and CS suggest caution when generalizing the results of longer-term VLNC cigarette studies from CS to SS. First, despite reporting similar smoking rates and nicotine dependence levels at enrollment, SS in this study had higher levels of smoke intake from usual-brand and VLNC cigarettes than CS during the 5-hr controlled administration periods. This finding is consistent with previous reports from several laboratories that SS smoke more intensely than CS (Tidey, Rohsenow, Kaplan, & Swift, 2005; Tidey et al., 2008, Weinberger et al., 2007, Williams et al., 2005, 2011). Although the functional significance of their higher smoking intensity is unknown, it supports the hypotheses that the neuropathology of schizophrenia confers stronger reinforcing effects of nicotine in SS (Novak et al., 2010) and that beneficial cognitive effects of nicotine contribute to more intense tobacco use in SS (Martin & Freedman, 2007). Their more intense smoking of VLNC cigarettes in this study suggests that SS could be more prone to compensatory smoking than CS. Notably, we found that 42 mg transdermal nicotine reduced VLNC total puff volume in both SS and CS during the 5-hr controlled administration periods. This is consistent with findings from longer-term studies in nonpsychiatric smokers indicating that nicotine replacement reduces compensatory smoking (Becker et al., 2008; Donny & Jones, 2009).

Another difference between SS and CS that is relevant to the potential success of a treatment approach incorporating VLNC cigarettes is that SS were less sensitive to the effects of VLNC cigarettes on smoking habit withdrawal severity. This finding indicates that VLNC cigarettes substituted less effectively for normal-nicotine content cigarettes in SS compared with CS, and suggests that SS could be less compliant with longer-term VLNC cigarette use. On the other hand, the finding that SS found VLNC cigarettes to be as effective as their usual brand in relieving cigarette craving suggests that, if they are compliant with longer-term VLNC cigarette use, these cigarettes may be an effective method of reducing their craving for normal-nicotine content cigarettes.

Smith and colleagues have conducted two previous studies of acute VLNC cigarette effects in SS (Smith et al., 2001, 2002). The initial study compared the effects of VLNC and high-nicotine content cigarettes on schizophrenia symptoms in 15 SS who underwent 6–12 hr smoking abstinence. Pre- versus postsmoking assessments indicated that smoking either VLNC or high-nicotine content cigarettes decreased negative symptoms to a similar extent; positive symptoms were unchanged (Smith et al., 2001). A subsequent study by this group examined the separate and combined acute effects of sensorimotor (VLNC or high-nicotine cigarettes) and nicotine replacement (nicotine or placebo nasal spray) on schizophrenia symptoms and cognitive performance in 31 SS who underwent 10–12 hr smoking abstinence (Smith et al., 2002). Consistent with their previous report, both VLNC and high-nicotine cigarettes improved negative symptoms, although the high-nicotine cigarette decreased negative symptoms to a greater extent, and positive symptoms were not affected. Cigarette type did not differentially affect cognitive task performance. In contrast, nicotine nasal spray improved cognitive performance to a greater extent than did placebo spray, but spray type did not differentially affect negative symptom levels. The current study replicates the findings of Smith et al. (2001, 2002) that acute use of VLNC cigarettes without concurrent nicotine replacement does not appear to adversely impact psychiatric symptoms.

Taking the above considerations into account, it is clear that the next step of this line of research should be to examine a longer period of VLNC cigarette use on measures of compliance, nicotine intake, carcinogen exposure, cognitive functioning, and psychiatric symptoms in SS. Given that cigarette craving levels and number of cigarettes smoked per day were reduced by

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approximately one-third in nonpsychiatric smokers after 6 weeks of VLNC smoking (Hatsukami, Kotlyar, et al., 2010), a 6-week period of VLNC cigarette use could provide an indication of whether this strategy may be effective in SS. Reducing the nicotine content of cigarettes to nonaddictive levels has been proposed as a public health approach for significantly reducing smoking-related morbidity and mortality in the United States (Benowitz & Henningfield, 1994; Hatsukami, Perkins, et al., 2010). The adoption of this strategy is now possible due to the passage of the 2009 Family Smoking Prevention and Tobacco Control Act, which gave the Food and Drug Administration the authority to reduce the level of nicotine in cigarettes. The findings of the current study suggest that this strategy may have potential for reducing smoking-related morbidity and mortality among SS. However, it is essential to examine the effects of VLNC cigarettes in SS over a longer period of time before this strategy can be fully considered.

Funding

This work was supported by National Institutes of Health grants R01DA14002 and P50DA031659 (JWT) and a Senior Research Career Scientist Award from the Department of Veterans Affairs (DJR).

Declaration of Interests

The authors have no conflicts of interest to report.

Acknowledgments

We thank Laura Dionne and Jennifer Schmidlin for their assistance with data management; Amy Adolfo Signore, Vera Mayercik, Netesha Reid, and Emily Xavier for their assistance with data collection; and the individuals who participated in this study for their contributions. This work was conducted at the Brown University Center for Alcohol and Addiction Studies, Providence, RI. Portions of these data were presented at annual meetings of the College on Problems of Drug Dependence and the Society for Research on Nicotine and Tobacco.

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Trial record **1 of 1** for: NCT01250301
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De-nicotinised Cigarettes Study (Denic)

This study has been completed.

Sponsor:
Queen Mary University of London

Collaborator:
Pfizer

Information provided by (Responsible Party):
Hayden McRobbie, Queen Mary University of London

ClinicalTrials.gov Identifier:
NCT01250301

First received: November 29, 2010
Last updated: July 16, 2012
Last verified: July 2012
History of Changes

Full Text View | **Tabular View** | **No Study Results Posted** | Disclaimer | How to Read a Study Record

Purpose

The aim of this study is to determine if using a behavioural replacement for smoking (de-nicotinised cigarettes; DNCs), in addition to standard treatment during the first two weeks after the target quit date, can reduce urges to smoke over the first 4-weeks of abstinence. Two hundred smokers who want to quit will be recruited from the community. They would all receive standard smoking cessation treatment from the NHS Stop Smoking Service (NHS SSS), which uses a combination of stop smoking medication (e.g. nicotine replacement therapy, Champix) and motivational support. On their target quit date, participants would be randomised to receive behavioural replacement (i.e. use of de-nicotinised cigarettes) plus standard treatment for the first two weeks of their quit attempt, or to continue with standard treatment alone. De-nicotinised cigarettes are similar to standard cigarettes except that they do not deliver nicotine to the smoker. Participants will rate their urges to smoke at each week. Standard NHS SSS measures will also be taken in addition to user acceptability ratings and reactions to smoking cues.

The hypothesis is that complementing current NHS SSS treatments with de-nicotinised cigarettes, to address the non-nicotine factors associated with smoking and to help extinguish smoking behaviour, would result in lower urges to smoke than standard treatment alone.

Condition	Intervention
Smoking Cessation Tobacco Dependence	Other: De-nicotinised cigarettes + standard treatment Other: Standard Treatment

Study Type: Interventional
Study Design: Allocation: Randomized

Endpoint Classification: Efficacy Study
Intervention Model: Parallel Assignment
Masking: Open Label
Primary Purpose: Treatment

Official Title: Complementing Current NHS Stop Smoking Service Treatments for Smokers With Behavioural Replacement: The Role of De-nicotinised Cigarettes

Resource links provided by NLM:

MedlinePlus related topics: Quitting Smoking Smoking

U.S. FDA Resources

Further study details as provided by Queen Mary University of London:

Primary Outcome Measures:

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- Urges to smoke in first week of abstinence [Time Frame: 1 week] [Designated as safety issue: No]

  Differences in urges to smoke in the first week of abstinence between participants who receive a behavioural replacement (DNCs) plus standard NHS SSS treatment, with those who receive standard NHS SSS treatment alone, will be measured by the Mood and Physical Symptoms Scale (MPSS; West & Hajek, 2004).

Secondary Outcome Measures:

- Change in ratings of tobacco withdrawal symptoms, measured by the MPSS [Time Frame: First 4 weeks of abstinence (from baseline- 4 weeks post-quit)] [Designated as safety issue: No]
- CO-validated continuous abstinence rates [Time Frame: 4-weeks] [Designated as safety issue: No]

  CO-validated continuous abstinence rates will be measured 1-4 weeks following the target quit day in the standard treatment condition and 1-4 weeks following cessation of DNCs in the DNC condition.

- Acceptability and user reactions to DNCs [Time Frame: 2 weeks] [Designated as safety issue: No]
- Urges to smoke 24 hours after quitting, measured by the MPSS [Time Frame: 24 hours] [Designated as safety issue: No]
- Profile of adverse effects up to 12 weeks post quitting [Time Frame: 12 weeks] [Designated as safety issue: Yes]
- Reactions to smoking cues [Time Frame: 12 weeks] [Designated as safety issue: No]

  The differences in reactions to smoking cues between the 2 groups will be measured with smoking-related images and by the Cue Exposure Subscale of the Wisconsin Inventory for Smoking Dependence (WISDM-68; Piper, et al., 2004).

Enrollment: 200
Study Start Date: July 2011
Study Completion Date: July 2012
Primary Completion Date: April 2012 (Final data collection date for primary outcome measure)

Arms	Assigned Interventions
Experimental: De-nicotinised cigarettes + standard treatment	Other: De-nicotinised cigarettes + standard treatment Participants will receive de-nicotinised cigarettes to use as required for 2 weeks after their quit day. These cigarettes are intended to provide a replacement for the behavioural and sensory aspects of smoking without supplying nicotine. Participants will also receive standard NHS Stop Smoking Service treatment. This is a combination of weekly behavioural support and medication (e.g. nicotine replacement therapy/varenicline). Other Names: • NHS Stop Smoking Service • De-nicotinised cigarettes
Active Comparator: Standard treatment	Other: Standard Treatment Participants will receive standard treatment from the NHS Stop Smoking Service.

Eligibility

Ages Eligible for Study: 18 Years and older
Genders Eligible for Study: Both
Accepts Healthy Volunteers: Yes

Criteria
Inclusion Criteria:

- 18 years or over
- Seeking treatment to stop smoking

Exclusion Criteria:

- Pregnant or breastfeeding
- Acute psychiatric illness

Contacts and Locations

Please refer to this study by its ClinicalTrials.gov identifier: NCT01250301

Locations

United Kingdom

Tobacco Dependence Research Unit
London, United Kingdom, E1 2JH

Sponsors and Collaborators

Queen Mary University of London

Pfizer

Investigators

Principal Investigator: Hayden McRobbie Queen Mary University of London

More Information

No publications provided

Responsible Party:	Hayden McRobbie, Dr. Hayden McRobbie, Queen Mary University of London
ClinicalTrials.gov Identifier:	NCT01250301 History of Changes
Other Study ID Numbers:	qmul200910
Study First Received:	November 29, 2010
Last Updated:	July 16, 2012
Health Authority:	United Kingdom: Research Ethics Committee

Keywords provided by Queen Mary University of London:
Smoking Cessation
Tobacco Dependence
De-nicotinised cigarettes

Additional relevant MeSH terms:
Smoking
Tobacco Use Disorder
Habits
Substance-Related Disorders
Mental Disorders

ClinicalTrials.gov processed this record on September 19, 2013