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

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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 has 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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Keywords

nicotine; smoking; placebo; abstinence; subjective ratings; conditioned reinforcement

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.*, 2003, Rose *et al.*, 2000, Rose *et al.*, 2010). Reinforcement from smoking likely occurs as the result of a combination 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.*, 2003, Rose *et al.*, 2000, 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 postmortem 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, Branam 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

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.

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 during 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 questionnaires 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" 100s; 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; < 0.05mg 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

Experiment 1: Device Validation—The impact of the smoking device on the time-courses of smoking-related subjective ratings, changes in physiological measures, and plasma nicotine levels, was assessed using repeated measures 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 outcomes. 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 ratings 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.

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 reinforcers, 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 baseline 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 factors. 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 differences 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 including heart rate ($F(1,7) < 0.001$, $p = 0.989$,

Fig. 1B), systolic blood pressure (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 compared to outcomes from sessions where cigarettes were smoked outside 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 significant 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 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", $F(1,9) = 32.325$, $p = 0.001$, Fig. 2A; "Feel Drug Effects", $F(1,9) = 25.998$, $p = 0.001$, Fig. 2B; "Like Drug", $F(1,9) = 35.552$, $p = 0.001$, 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; $p = 0.001$, 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; $p = 0.001$, 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; $p = 0.001$, Fig. 2C). Cigarette type significantly impacted positive subjective ratings of "High" ($F(1,9) = 12.474$; $p = 0.001$) and "Feel Drug" ($F(1,9) = 14.418$; $p = 0.001$), with pairwise comparisons indicating that nicotine cigarettes increased ratings more than placebo cigarettes ("High" mean difference = 1.043, 95% CI = 0.463–1.623, $p = 0.001$; "Feel Drug" mean difference = 1.339, 95% CI = 0.646–2.032, $p = 0.001$; "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 (Figs. 3 A–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 ($F(1,13) = 14.885$, $p = 0.001$; Fig. 3A), significantly greater drug liking, ($F(1,9) = 7.949$, $p = 0.005$, 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 ($F(1,9) = 18.626$, $p = 0.001$, 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 $10.288 - 2.034$; $p = 0.001$, Fig. 2D). Time was also found to have a significant main effect on ratings of “Anxious” ($F(1,9) = 4.467$, $p = 0.001$, 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” ($F(1,9) = 11.870$, $p = 0.001$), 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” $F(1,9) = 4.467$, $p = 0.001$; “Anxious” $F(1,9) = 16.032$, $p = 0.001$; “Irritable” $F(1,9) = 3.105$, $p = 0.001$). There was no significant interaction between cigarette type and abstinence state on any ratings of negative reinforcement in this relatively 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 subjective 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 significant 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 consistent 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 affect in smokers (Perkins *et al.*, 2010). Conversely, conditioned reinforcers appear to play a relatively 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 compared to non-abstinence ($F(1,9) = 12.973$, $p = 0.001$, Fig. 3D) irrespective 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” $F(1,9) = 25.544$, $p = 0.001$, Fig. 3E; “Irritable” $F(1,9) = 12.292$, $p = 0.001$, 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 interaction between cigarette type and abstinence state was found for subjective 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 condition = 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 considered, nicotine content had a significant main effect on all three ratings of positive reinforcement and no main effect on any of the three ratings of negative reinforcement. These data suggest that nicotine, 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 reinforcement: “Crave”, “Anxiety”, and “Irritability”. In normalized analyses of time points during which conditioned reinforcers were present, abstinence state exerted significant effects on both positive and negative 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 cigarette of the day.

Taken together, these data provide evidence that the presence of nicotine has a greater impact on positively reinforcing effects of cigarette 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 hemodynamic changes in brain in order to better understand the neuronal substrates mediating the subjective effects of nicotine and placebo smoking.

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Highlights

- An MRI-compatible smoking device was developed and tested.
- The device delivered the same amounts of nicotine as typical smoking.
- Physiological effects and ratings were the same smoking with or without the device.
- Cigarette type impacted ratings of positive more than negative reinforcement.
- Smoking abstinence impacted both ratings of positive and negative reinforcement.

Figure 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.

Figure 2. Full time courses of smoking induced subjective ratings

All four conditions analyzed in the $2 \times 2 \times 9$ 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: * $p < 0.05$) Abstinance 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 abstinance 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.

Figure 3. Subjective ratings in the presence of conditioned reinforcers

All four conditions analyzed in the $2 \times 2 \times 3$ 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 (* $p < 0.05$). 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).