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PHARMACODYNAMICS

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Comparative effects of tobacco smoking and nasal nicotine

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Abstract *Objective*: Compare the electroencephalographic (EEG) and cardiovascular effects of tobacco smoking and nasal nicotine in the same subjects.
Methods: Eleven volunteer smokers were studied after >10 h of overnight tobacco deprivation. Quantitative EEG was used to measure brain electrical changes produced by four different treatments. Each subject smoked a low (0.08 mg) and average nicotine (1 mg) yield cigarette on one test day and received placebo and nicotine nasal spray (0.5 mg/spray) on a second day in a counterbalanced design. EEG activity was measured from 16 scalp electrodes and analyzed as *delta, theta, alpha$_1$, alpha$_2$, beta$_1$,* and *beta$_2$* frequency bands. Heart rate (HR), blood pressure (BP), and plasma venous nicotine concentrations (VNC) were monitored during both sessions. EEG data from all 16 channels at each of six frequencies were compared over 10 min using repeated measures ANOVA analysis. Changes in HR, BP, and VNC from baseline were compared using ANOVA followed by *post hoc* Scheffe's test.
Results: Smoking an average nicotine delivery cigarette resulted in highly significant decreases in *alpha$_1$* activity, significant increases in *alpha$_2$* activity, and significant increases in both HR and VNC compared to all other conditions.
Conclusion: When smokers are allowed to pace themselves, cigarette smoking is far more effective than nasal nicotine in activating the EEG and increasing HR and VNC. This lack of equivalent physiological effects may explain the low success rate when nicotine nasal spray is used by those trying to quit smoking.

Keywords Brain · Electrophysiology · Nicotine · Tobacco

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Introduction

A large body of basic neuroscience data indicates tobacco addiction involves nicotine [1], which releases the neurotransmitter dopamine (DA), similar to other drugs of abuse [2]. Nicotine nasal spray is a delivery system, which allows more rapid increases in venous blood levels of nicotine than with other nicotine replacement therapies. However, treatment success is only about 18% of smokers that remain abstinent after one year with nicotine nasal spray versus 8% with placebo [3]. The high blood levels of nicotine achieved rapidly upon smoking a cigarette probably account for the greater objective and subjective effects when compared to more slowly released forms of nicotine [4, 5].

In the present study, quantitative EEG was used to measure brain electrical changes produced by four drug conditions: a low nicotine yield cigarette, an average nicotine yield cigarette, commercially available nicotine nasal spray, and a placebo nasal spray containing oleoresin of pepper.

Materials and Methods

Subjects

The study protocol was approved by the University of Michigan Institutional Review Board. Twenty-five healthy adult male (13) and female (12) cigarette smokers (>15 to 40 cigarettes per day), ranging in age from 18–44 years, and free from medication use (except for females taking oral contraceptives) were recruited through advertisements placed on the University of Michigan Central Campus and in the University of Michigan Hospitals.

Exclusion/inclusion criteria

Subjects with any physical or mental disease were excluded based on medical history, physical exam, or serum electrolyte, liver enzymes, creatinine, BUN, and CBC results. Those with a history of psychotic, manic or bipolar disorder were also excluded. Subjects who had a history of psychoactive substance abuse disorder within the past six months, or who revealed evidence of recent use of abused substances in a urine toxicology screen were excluded. Persons who were currently taking medications (except for females taking oral contraceptives) and those who had taken antidepressant, anxiolytic, or antipsychotic medications within the past 6 months were excluded. Candidates who seemed eligible on the basis of a preliminary telephone screen were invited to a screening interview, at which time the study was explained and informed consent obtained. A brief physical examination, including height, weight, blood pressure, and heart rate was conducted. Laboratory tests on blood including complete blood count, electrolytes, liver function tests, urinalysis and urine toxicology screen were obtained for screening purposes. Patients were also assessed for degree of nicotine dependence using the Fagerström Test for Nicotine Dependence (FTND) [6].

On the day of a test session, each subject reported to the testing laboratory at 0800. A Vitalograph Breath CO monitor (Vitalograph USA, Lenexa, Kansas) was used to determine the parts per million (ppm) of exhaled carbon monoxide (CO). Alveolar CO < 10 ppm was one of the criteria of tobacco abstinence.

Experimental design

Subjects who passed screening procedures were scheduled for two testing sessions on different days at least one week apart. None of the subjects and only one of the investigators knew the order of the treatments. During one test session, subjects smoked a low nicotine yield (0.08 mg) cigarette followed 1 h later by an average nicotine yield (1.0 mg) cigarette. During the other test session the subject received placebo nasal spray followed by nicotine nasal spray. The order of testing sessions (cigarettes versus sprays) was randomized. Prior to both study sessions, subjects were asked to abstain from cigarette smoking overnight > 10 h before reporting for study the next morning. They were also asked not to drink any alcohol for one day prior to the study session, and not to drink any caffeinated beverages on the morning of the study.

Both research cigarettes were kindly provided by Philip Morris, U.S.A. The nicotine nasal spray was delivered using a commercially available nicotine spray (McNeil Laboratories), which provides a metered dose of nicotine solution equivalent to 0.5 mg per spray. The active placebo oleoresin of pepper spray was provided by Dr. K.O. Fagerström. An attempt was made to acclimate the subjects to the nicotine spray by showing them the correct method of administering the spray. Subjects were also given the nicotine spray to practice with and use at home prior to the study sessions. If subjects were not able to tolerate the entire dose of nicotine spray, they received a lower dose. The number of sprays administered varied from two to a maximum of six, as tolerated. The placebo spray contained oleoresin of black pepper that partially simulates the nasal irritation of nicotine.

A heparin lock was placed in a forearm vein to facilitate blood drawing. Immediately prior to smoking a cigarette or receiving nasal spray, a 5 ml baseline blood sample was obtained. Another sample was obtained 10 min following the initiation of smoking or administration of nasal spray. Blood samples were placed on ice, centrifuged, and the plasma stored at –20°C until analysis. Samples were analyzed for nicotine concentration using the method of Hariharan et al. [7].

The subjects rested in a supine position in a comfortable recliner in the experimental smoking room. The EEG procedure utilized 16 cortical sites for monopolar recordings linked to $A_1$and A_2 as the reference lead per the 10–20 International System [8]. An electrode cap (Electrode Cap International, Eaton, OH, USA) was placed on the subject's head with Grass electrode paste applied to each electrode. Electrode salt paste was used to keep electrode resistance below 5000 ohms to both ear lobes as reference. The subjects smoked, at their usual rate and depth of inhalation, a low-nicotine cigarette for 5 min maximum, or received placebo nasal spray (2–6 sprays). EEG recordings (with eyes closed) were taken before and at 3, 6, and 10 min from the start of smoking the low-nicotine cigarette or receiving the placebo nasal spray. One hour later, the subject smoked an average-nicotine cigarette for 5 min or received nicotine nasal spray (2–6 sprays). EEG recordings were again obtained at baseline, 3, 6, and 10 min. HR and BP were monitored at the same time as the EEG.

Data analysis

The EEG recordings taken from F_7, F_8, T_3, T_4, T_5, T_6, Fp_1, Fp_2, F_3, F_4, C_3, C_4, P_3, P_4, O_1, and O_2 were recorded on channels 1–16 of a Grass electroencephalograph (Model 8–24D). Computer analysis of the electrophysiological data was completed offline by the software package RHYTHM 7.1 (Stellate Systems, Quebec, Canada) utilizing a Zenith 386/25 microcomputer. Analog to digital conversion of the EEG signal was done for the maximum number of artifact-free epochs in each recording period. The digitized data underwent fast Fourier transformation to determine the frequency characteristics in each of the 6 bandwidths; 1–3.75 Hz (*delta*), 4–7.5 Hz (*theta*), 7.75–10 Hz ($alpha_1$), 10.25–12.5 Hz ($alpha_2$), 12.75–20 Hz ($beta_1$), and 20.25–31 Hz ($beta_2$). Analyses of the EEG data were accomplished using a within subjects ANOVA for repeated measures. Absolute EEG amplitudes, within the six frequency ranges, were compared across the four drug conditions at baseline, 3, 6, and 10 min using the PROC MIXED program in SAS Version 8.1 for Windows (SAS Institute Inc., Cary, NC, USA). *Post hoc* comparisons were conducted using contrasts; *alpha* was adjusted to 0.0083. The HR, BP, and nicotine plasma concentration differences were compared across the four drug conditions using ANOVA followed by *post hoc* Scheffe's test. Figure 1 depicts the trial design.

Results

The selected study population consisted of 7 male and 6 female subjects who completed both days of testing. However, baseline nicotine blood levels indicated that two female subjects had unacceptably high baseline nicotine levels (> 10 ng/ml), indicating these two volunteers were probably noncompliant. These subjects were removed from the data analyses. The results are based on the remaining 11 subjects (7 M, 4 F). The mean age for male subjects was 26.3 years (19–36 years), and for female subjects 26.3 years (18–32 years). Eight subjects were Caucasian, 1 African-American, 1 Korean, and 1 East-Indian. The average Fagerström Test for Nicotine Dependence score was 3.6 (range 0–6); average score for men was 3.4 (range 0–6) and for women 4.0 (range 2–5).

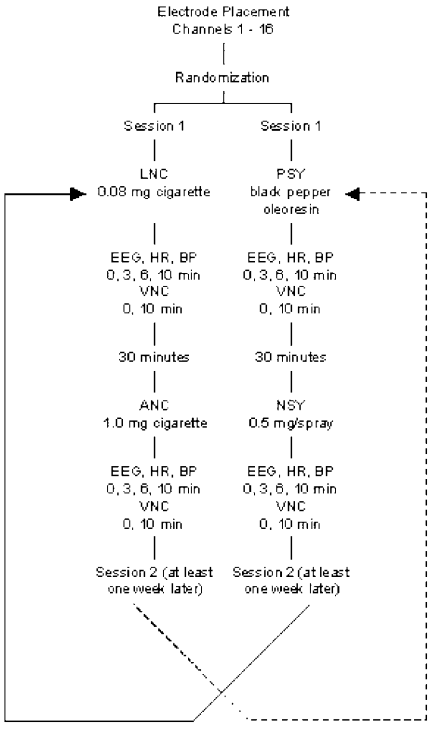

Fig. 1. Experimental design. Symbols used in this and/or the following figures: *PRE*, before any treatment >10 h overnight tobacco deprivation; *LNC*, after smoking a low nicotine yield cigarette; *ANC*, after smoking an average nicotine yield cigarette; *NSY*, after nicotine nasal spray; *PSY*, after placebo oleoresin of pepper spray; *VNC*, plasma venous nicotine concentrations

Plasma venous nicotine levels

ANOVA followed by *post hoc* Scheffe's test revealed significantly higher nicotine concentrations at 10 min following the smoking of an average nicotine yield cigarette. In this small sample, smoking an average nicotine cigarette resulted in much higher nicotine concentrations in men than women, probably reflecting differences in smoking style. Interestingly, the administration of nicotine nasal spray did not cause equivalent elevations of plasma nicotine in most subjects. Smoking an average nicotine yield cigarette produced the largest increase in plasma nicotine in both genders, as illustrated in Fig. 2. In view of the small N, the gender differences were not significant and the data were pooled. Both the low nicotine yield cigarette and the nicotine nasal spray produced relatively minor increases in plasma nicotine levels.

Electroencephalographic effects

Within subjects repeated measures ANOVA revealed an interaction between drug condition (average nicotine cigarette, low nicotine cigarette, nicotine nasal spray, placebo nasal spray) and time (3, 6, and 10 min), which was statistically significant ($p = 0.0083$) in most channels, especially within the *alpha$_1$* frequency range (see Table 1. There was a significant decrease in *alpha$_1$* activity at 3 min across all 16 channels and a significant increase in *alpha$_2$* activity at 10 min in 8 channels, following the smoking of an average-nicotine cigarette. The reduced amplitude seen in the *alpha$_1$* frequency band at 3 min was especially pronounced in channels 5, 6, and 9–14 (T_5, T_6, F_3, F_4, C_3, C_4, P_3 and P_4). The increased amplitude seen in the alpha$_2$ frequency band at 10 min occurred mostly in channels 1, 2, and 8 (F_7, F_8, and Fp_2). Figure 3 summarizes the statistically significant differences in *alpha$_1$* and *alpha$_2$* activity seen at 3 and 10 min, respectively. Smoking an average nicotine yield cigarette (ANC) produced a marked decrease in *alpha*$_1$ activity with recovery to baseline in 10 min. In contrast, the marked increase in *alpha$_2$* activity was maximal

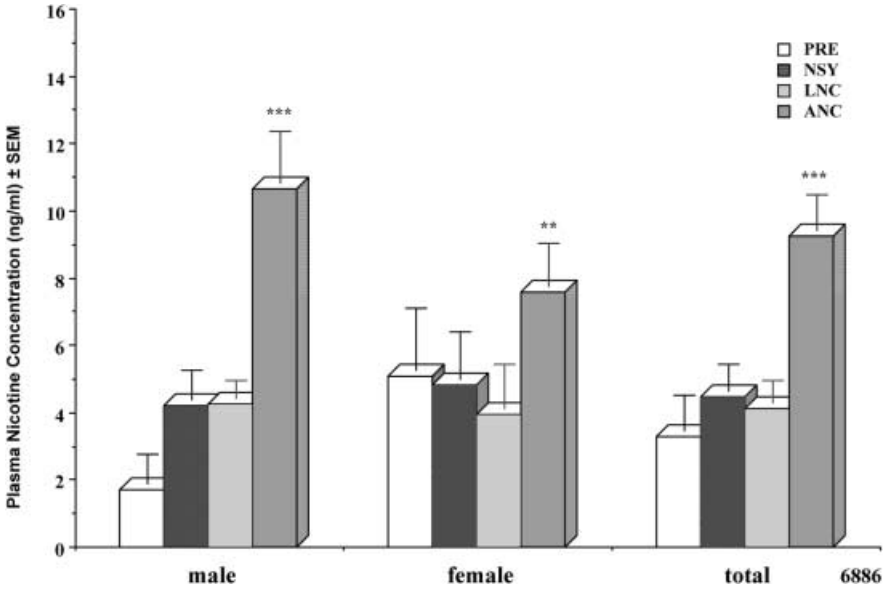

Fig. 2. Effects of cigarette smoking and nicotine nasal spray on plasma nicotine levels. Note statistically significant differences. $^{**}p < 0.01$, $^{***}p < 0.001$

Table 1. Statistically significant ($p = 0.0083$) differences between smoking an average nicotine yield cigarette and all other drug conditions: low nicotine yield cigarette (L), nicotine nasal spray (N), and placebo nasal spray (P)

EEG channel	*Delta* 1–3.75 Hz 10 min decrease	*Theta* 4–7.5 Hz 3 min decrease	*Alpha*$_1$ 7.75–10 Hz 3 min decrease	*Alpha*$_2$ 10.25–12.5 Hz 6 min increase	*Alpha*$_2$ 10.25–12.5 10 min increase
1		L	L, N		L, N, P
2		N	L, N	P	L, N, P
3			L, N	N, P	N, P
4			N	P	P
5	L	L	L, N, P		
6		N	L, N, P		
7			N	P	L, P
8			L, N	P	L, N, P
9	L	N	L, N, P	P	L, P
10	L	N	L, N, P	P	P
11		N	L, N, P		
12	L	N	L, N, P		
13		N, P	L, N, P		
14	L	N, P	L, N, P		
15			L, N		
16	L		L		

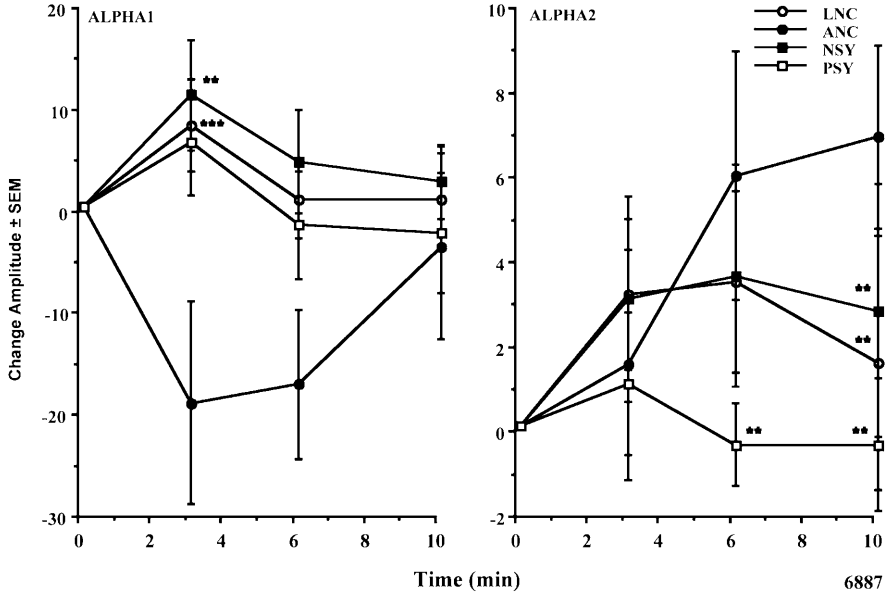

Fig. 3. Effects of cigarette smoking and nicotine nasal spray on changes in EEG *alpha* frequency. The EEG *alpha* changes for two channels (*alpha*$_1$ channel 15 and *alpha*$_2$ channel 8) are plotted after various treatments. Statistically significant differences from ANC are noted. $^{**}p < 0.01$, $^{***}p < 0.001$

10 min after ANC. Both low nicotine yield cigarettes (LNC) and nicotine nasal spray (NSY) produced similar but much smaller changes. The placebo nasal spray was relatively ineffective compared to the other treatments. Differences found in the *delta, theta*, and *beta*$_1$ frequency bands, among the four drug conditions, were not as pronounced or as consistent as those seen in the *alpha*$_1$ and *alpha*$_2$ frequency bands. There were no statistically significant differences found in the *beta*$_2$ band among any of the drug conditions.

Effects on heart rate

Repeated measures ANOVA followed by *post hoc* Scheffe's test revealed significantly higher HR after smoking an average nicotine yield cigarette at 3, 6, and 10 min. The mean change in heart rate from baseline (ΔHR) over time is plotted in Fig. 4. The differences in HR after smoking a low nicotine yield cigarette and nicotine nasal spray were not statistically significant. The peak HR increases occurred at 3 min after smoking or nicotine nasal spray. Mean increases $\pm$ SD in HR from baseline to 3 min after smoking an average and low nicotine yield cigarette and after receiving nicotine and placebo nasal spray were 19.9 ± 8.9, 6.9 ± 8.3, 8.0 ± 7.2 and 0.8 ± 4.1 beats/min, respectively. The maximum HR recorded after smoking an average nicotine yield cigarette was 116 beats/min. Smoking an average nicotine yield cigarette (ANC) produced the greatest mean change in HR, whereas a low nicotine yield cigarette (LNC) and nicotine nasal spray (NSY) produced similar, small increases in HR. The placebo oleoresin of pepper had no effect. Surprisingly, there were no statistically significant differences in BP found between any of the nicotine conditions.

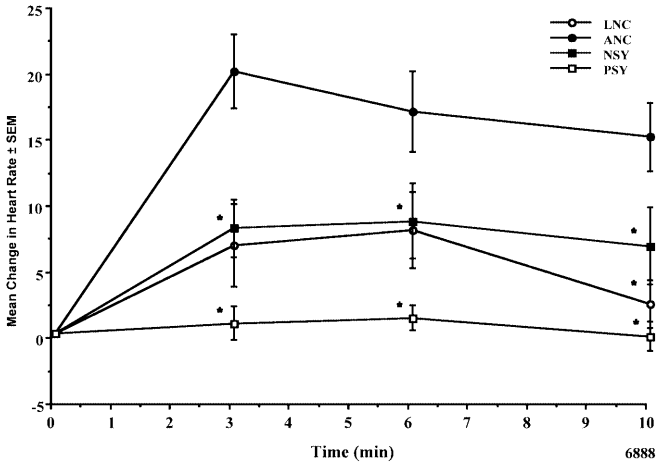

Fig. 4. Effects of cigarette smoking and nicotine nasal spray on heart rate. Note that after smoking an average nicotine yield cigarette (ANC) both the heart rate increase (this figure) and the increase in *alpha*$_2$ (Fig. 3) persist for at least 10 min. Statistically significant differences from ANC are noted $*p < 0.05$

Discussion

The principal finding of this study was that smoking an average nicotine yield cigarette resulted in significantly different physiological effects in comparison with self-paced administration of nicotine nasal spray. Smoking an average nicotine cigarette was accompanied by a shift to higher brain wave frequencies; most notably a shift from *alpha*$_1$ to *alpha*$_2$ activity across 10 min. Furthermore, smoking an average nicotine yield cigarette resulted in significant increases in both heart rate and plasma nicotine concentrations as compared to nasal nicotine administration.

It has been well documented that the administration of nicotine and its deprivation in smokers results in changes in EEG activity [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. Various periods of smoking deprivation have resulted in increased lower frequency bands power [14, 15, 18, 19], while tobacco smoking produces a shift to higher frequencies in deprived smokers [9, 10, 11, 12, 13, 14, 16, 17]. The current investigation corroborates earlier studies that tobacco smoking causes a shift from lower to higher brain wave activity.

Smoking tobacco has consistently been shown to increase heart rate [9, 10, 11, 20, 21, 22]. Smoking an average nicotine yield cigarette resulted in significantly higher heart rates in our subjects, which corresponded to significantly higher concentrations of plasma nicotine when compared with either low nicotine yield cigarettes or nicotine nasal spray. This is not surprising since increases in heart rate are correlated with increasing plasma nicotine levels [11, 20, 21].

Tobacco smokers obtain far more nicotine by smoke inhalation than they are able to tolerate by nasal spray. The venous plasma nicotine levels observed in our study following the administration of nicotine nasal spray are lower than those reported previously with plasma levels between 5 and 12 ng/ml [23]. When given as a single 1 mg dose, similar to the amount of nasal nicotine delivered in the current investigation, mean peak venous nicotine concentrations of 8.1 ng/ml were seen, which occurred at a peak rise time of 11.5 min [3]. The lower plasma nicotine levels in our population were due to the smokers' strong dislike for the nasal spray. Subjects in our sample found the nicotine nasal spray very irritating and obviously did not use it optimally. This is consistent with the literature, which indicates that subjects often complain of nasal irritation, throat irritation, sneezing, runny nose, watery eyes, and coughing [3, 24, 25]. Adverse effects significantly contribute to the lack of popularity of this preparation within the general population of those trying to quit smoking, especially when emotional support is not available as it is during clinical trials.

The difference in EEG effects found between nasal nicotine and average nicotine yield cigarettes in the present study are probably due to differences in total nicotine intake. Changes in EEG activity are correlated with plasma nicotine levels [11, 26]. Kadoya et al. [11] found a statistically significant decrease in *delta* activity with an increase in venous plasma nicotine of 15 ng/ml or more, while a decrease in *alpha*$_1$ and increase in *beta* activity were found with plasma nicotine increases of 10 ng/ml or more.

While this experimental design allowed comparison of physiological parameters between low nicotine and average nicotine yield cigarettes, and also between placebo and nicotine nasal spray on the same experimental day, for each procedure the comparisons between cigarettes and nasal spray were not conducted on the same day. Baseline conditions did not differ significantly between the days on which subjects received cigarettes or nasal spray. A major limitation of this study is the lack of predetermined dose of either the cigarettes or nasal spray (*i.e.,* the subjects were allowed to decrease their dose if intolerable). However, this study design reflects what patients do in "real-life" situations. Nicotine nasal spray would probably be more effective clinically if each smoker was told he or she must try to gradually increase the number of sprays to a maximum equivalent to the absorbed dose of nicotine they achieve by smoking a cigarette. Reinforcement and addiction to nicotine are thought to be caused by rapid delivery of nicotine to the brain [27]. This study provides direct evidence that the higher blood levels of nicotine achieved rapidly upon smoking a cigarette account for the greater objective and subjective effects when compared to more slowly released forms of nicotine.

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