Regional Cerebral Blood Flow Responses to Smoking in Tobacco Smokers After Overnight Abstinence

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Abstract

OBJECTIVE: The purpose of the study was to determine the effects of cigarette smoking on brain regional function in a group of chronic smokers by using cerebral blood flow (CBF) measures and positron emission tomography (PET). METHOD: Nineteen tobacco smokers were studied after about 12 hours of smoking abstinence. Regional CBF (rCBF) measures were obtained with PET and $[^{15}]$O$_2$H$_2$O in six consecutive scans. Two average-nicotine-yield (1.0 mg) and one denicotinized (0.08 mg) research cigarettes with similar tar yields (9.5 mg and 9.1 mg, respectively) were smoked in a double-blind design, preceded and followed by baseline scans. The rCBF effects of smoking were compared to baseline measures and between cigarettes, as well as to subjective ratings of craving for cigarettes. RESULTS: Smoking the first cigarette of the day resulted in increases in rCBF in the visual cortex and the cerebellum and reductions in the anterior cingulate, the right hippocampus, and the ventral striatum, including the nucleus accumbens. Cigarette craving scores correlated with rCBF changes in the dorsal anterior cingulate and right hippocampus. Less pronounced effects were observed with the second cigarette and the denicotinized cigarette. CONCLUSIONS: Smoking affects rCBF not only in areas of the brain rich in nicotinic cholinergic receptors but also in areas implicated in the rewarding effects of drugs of abuse. Furthermore, craving for a cigarette in chronic smokers was correlated with rCBF in the right hippocampus, an area involved in associating environmental cues with drugs, and in the left dorsal anterior cingulate, an area implicated in drug craving and relapse to drug-seeking behavior.
Introduction

Tobacco is the most widely abused substance, and evidence suggests that abuse is linked to the nicotine content of tobacco. Nicotine produces tolerance, dependence, and withdrawal symptoms in a manner similar to other substances of abuse. The most convincing evidence of the reinforcing properties of nicotine comes from studies showing that nicotine is intravenously self-administered in animal models (e.g., references 1, 2) and human smokers (3). Furthermore, studies suggest that nicotine acts similarly to other abused substances at neurobiological levels. For example, repeated nicotine injection results in behavioral sensitization similar to that induced by psychostimulants such as cocaine and amphetamine (4).

The behavioral and reinforcing effects of nicotine are thought to be mediated through the activation of the mesolimbic dopaminergic pathway (5, 6), an effect shared with other addictive drugs (e.g., references 7, 8). Like other substances of abuse, nicotine increases extracellular dopamine levels in the nucleus accumbens in rats (9) and humans (10). Furthermore, lesions of the mesolimbic dopaminergic pathway attenuate nicotine self-administration (6) and the locomotor activating effects of nicotine (11).

The effects of nicotine on human brain functional responses, measured by changes in regional cerebral blood flow (rCBF), metabolism, or blood oxygenation level, have been reported by a number of investigators. However, the results have been equivocal in terms of both the direction and localization of changes. In a functional magnetic resonance imaging (fMRI) study of smokers, the effect of intravenous nicotine on the resting brain was examined shortly after subjects smoked freely throughout the day (12). Dose-dependent increases in neuronal activity were noted in a number of widely distributed brain regions, including areas of the cingulate, prefrontal, temporal, and occipital cortices; putamen; nucleus accumbens; thalamus; hypothalamus; and amygdala. Similarly, in a positron emission tomography (PET) study, Nagata and colleagues (13) found increases in rCBF in the frontal lobes and cerebellum after cigarette smoking. In a series of studies investigating the effect of nasal nicotine spray on rCBF and regional metabolic rate of glucose in the resting brain of habitual smokers after 12 hours of abstinence, increases were found in the frontal, cingulate, and visual cortices and in the thalamus and cerebellum. Reductions in activity were observed in the anterior temporal and insular cortex and in the amygdala (14–16). Those results led to the hypothesis that the reductions in limbic activity during nicotine administration after overnight abstinence may have been due to the lowering of synaptic function in regions implicated in the drive to smoke or craving for nicotine. More recently, Rose and colleagues (17) also observed nicotine-induced rCBF changes in left hemisphere regions, including the ventral striatum, prefrontal cortex, anterior cingulate, and occipital cortex and dose-related decreases in the left amygdala after overnight abstinence.

Nicotine is known to mediate a number of cognitive and behavioral functions, such as memory, locomotion, antinociception, and appetite (18), and when subjects are performing cognitive tests, nicotine alters rCBF. Intravenous nicotine infusion during a psychometric task after 24 hours of smoking abstinence in smokers caused increases in rCBF in parieto-occipital regions, but reductions in the anterior cingulate cortex and cerebellum, with both types of changes correlating with plasma nicotine levels (19). During a working memory task, smokers (abstinent for 12 hours) and ex-smokers displayed different patterns of activation with nicotine gum versus placebo (20). Specifically, nicotine caused less overall activation in smokers, whereas it increased activation in ex-smokers. It is interesting to note that anterior cingulate activation decreased with nicotine in both groups. During a sustained attention task, transdermal nicotine was found to improve performance in smokers and increase task-induced brain activation in the parietal cortex, thalamus, and caudate (21). Some of the variability in the direction and localization of the results that have been reported may be due to the status of the subjects at the time of the study (e.g., time since last cigarette), as well as the brain regions examined and the mode of nicotine administration.

We conducted an rCBF PET study of the effects of smoking average-content nicotine cigarettes and denicotinized cigarettes in a group of 19 male and female chronic smokers after 12 hours of tobacco abstinence. We chose to directly study the effects of smoking a cigarette, as that route of administration is used by the majority of those who are addicted to nicotine. In addition, to specifically examine the effects of nicotine in the brain, we used a subtractive method with a control condition in which a low-nicotine cigarette was smoked. This design allowed us to control for nonnicotine aspects of smoking, as well as for other substances found in cigarette smoke that may affect cerebral perfusion, such as tar, carbon monoxide (CO), and carbon dioxide (CO₂). Finally, to investigate the specific effects of smoking the first cigarette of the day, versus any consecutive cigarette, we had the subjects smoke a second cigarette with average nicotine content, which was randomized in order with the denicotinized cigarette. We hypothesized that smoking would affect rCBF in a distributed network of brain regions, including direct effects in regions regulated by nicotinic receptors (i.e.,...
occipital cortex, thalamus) and in regions typically associated with the cognitive and reinforcing effects of drugs of abuse (i.e., limbic, paralimbic, and prefrontal cortical structures).

**Method**

**Subjects**

Nineteen healthy volunteers, eight men and 11 women ages 19–51 years (mean=27 years, SD=10) who smoked seven to 30 cigarettes per day (mean=15.8, SD=5.5), were recruited by advertisement. The subjects underwent physical and neurological examinations and reviews of medical history. Undiagnosed current or past psychiatric conditions were ruled out by a structured interview (22). Only subjects who had no current or past history of serious physical illnesses, neurological or psychiatric illness, substance dependence (except nicotine), or recent substance abuse (within the last year) and who had not taken psychoactive substances within the prior month were included. After the study was completely described to the subjects, written informed consent was obtained. The study was reviewed and approved by the Institutional Review Board for Human Subject Research and the Radioactive Drug Research Committee at the University of Michigan.

All subjects were medication free except for oral contraceptives. Women were screened for pregnancy by using the QuickVue, One-Step hCG-Urine Qualitative Detection test (Quidel Corp., San Diego) before inclusion in the study. Subjects who met the inclusion criteria completed the Fagerström test (23) to provide an assessment of the degree of tobacco dependence. The mean Fagerström score for participants was 3.6 (SD=2.0).

Subjects reported to the PET suite of the Nuclear Medicine Division at the University Hospital at 7:30 a.m. on the day of the study. Subjects were instructed to cease cigarette or tobacco use overnight, approximately 12 hours before the study, and their compliance was confirmed with breath CO testing. A sample of expired air was analyzed for CO in parts per million (ppm) by using a CO detector (Vitalograph Breath CO Model BC1349, Vitalograph Inc., Leneta, Kan.). Expired air CO levels greater than 10 ppm required an extensive reinterview to ascertain possible noncompliance with the no-smoking directive within the past 12 hours. All subjects appeared compliant with this requirement.

**Design**

Regional CBF was determined before and after smoking cigarettes with average nicotine content or cigarettes with low nicotine delivery. The two different types of research cigarettes were obtained through the courtesy of Dr. Frank P. Gullota and Ms. Cynthia S. Hayes of the Philip Morris Research Center, Richmond, Va. The nicotine-containing cigarette was prepared with unextracted tobacco (nicotine: 1.01 mg/cigarette; tar: 9.5 mg/cigarette). The low-nicotine cigarette was made with almost 100% extracted tobacco (nicotine: 0.08 mg/cigarette; tar: 9.1 mg/cigarette). Both cigarettes contained identical filter tips and were made from the same blend of tobacco with no flavors added. Thus, their tar content was almost identical (9.5 mg versus 9.1 mg), and only the amount of nicotine per cigarette was markedly different (1.01 mg versus 0.08 mg).

Subjects were encouraged to puff every 30 seconds for a total number of 10 puffs per cigarette. The volume of each puff was not controlled for, and subjects were instructed to smoke as they normally smoked. To account for interindividual variations in the plasma level of nicotine, arterial nicotine levels were obtained throughout the PET studies, as described later in the Method section.

The order of scans was as follows: scan 1, baseline; scan 2, after inhalation of first cigarette; scan 3, baseline; scan 4, after inhalation of second cigarette; scan 5, baseline; scan 6, after inhalation of the third cigarette. The first cigarette was always a nicotine-containing cigarette. The second and third cigarettes were a nicotine-containing cigarette and a denicotinized cigarette, the order of which was randomized and counterbalanced between subjects.

Visual analogue scales were administered at baseline and after each scan. Subjects were asked to rate, on a scale of 0 (not at all) to 10 (most ever), how they felt at that moment with regard to craving for a cigarette and the extent to which they felt relaxed, nervous, sick, and wakeful.

**PET Imaging**

Imaging was performed with a Siemens ECAT 931/08–12 PET scanner (Knoxville, Tenn.), which acquires 15 simultaneous planes with a separation of 6.75 mm between planes over a 10-cm field of view (reconstructed resolution: -10 mm at full width half maximum). Subjects were positioned in the scanner gantry so that the field of view included the top of the brain to the middle of the cerebellum; a 3-minute transmission scout view was used to confirm the positioning. Head position was maintained by soft restraints and was monitored by laser beams. EKG and systemic arterial blood pressure were monitored throughout the study (Series 7000 monitor,
Marquette Electronics, Inc., Milwaukee). Two intravenous and one arterial indwelling catheters were placed for radiotracer administration and blood sample removal. Arterial blood samples for nicotine were withdrawn at baseline (scan 1) and 3, 5, 10, 15, and 20 minutes after each scan in which a cigarette was smoked (scans 2, 4, and 6). The 15-minute and 20-minute interval blood draws after scans 2 and 4, therefore, took place during the baseline scans (scans 3 and 5), and those after scan 6 took place after scanning was completed but while the subject was still in the gantry. Blood samples were collected in standard 5-ml EDTA Vacutainer (Becton Dickinson, Franklin Lakes, N.J.) tubes and stored immediately on crushed ice. Immediately after completion of the study the samples were centrifuged and plasma aliquots were frozen at −20°C until analysis. Arterial samples were analyzed for nicotine by using high-performance liquid chromatography techniques (24) or a gas chromatographic-nitrogen detector assay (MEDTOX Laboratories Inc., St. Paul, Minn.).

For each scan, the subjects received 50 mCi [15O]H2O intravenously, and each of the six scans was run 12 minutes apart. Data acquisition began 5 seconds after the arrival of the labeled compound in the brain and continued for 60 seconds.

**Data Analysis**

Images were reconstructed by using a Parzen filter with a cutoff frequency of 0.45 cycles/projection element, and attenuation was corrected by a 10- to 15-minute transmission scan (68Ge source) obtained before the first scan. After reconstruction, image sets were coregistered to each other, reoriented to the intercommisural line, and nonlinearily warped to the International Consortium on Brain Mapping atlas stereotactic coordinates by using SPM 99 routines (25). Image data were then normalized to whole brain counts, as previously described (26).

To compensate for small residual anatomic variations across subjects and to improve signal-to-noise ratios, a three-dimensional Gaussian filter (full width at half-maximum of 6 mm) was applied to each scan. For each subtraction analysis, two-tailed t statistic values were calculated for each voxel by using SPM 99 (25) and the pooled variance across voxels (27). Areas of significant differences were detected with a statistical threshold that controls a type I error rate at p=0.05 for multiple comparisons, which is estimated with the Euler characteristic (28) based on the number of voxels in the gray matter and on image smoothness (29). Changes in rCBF were also deemed significant if they reached the p<0.05 threshold after the size of the region involved was accounted for (cluster-level comparison [30]). Findings are also reported for regions that did not reach the multiple comparison-corrected threshold if the changes observed were consistent with previous data from our group and others (e.g., increases in rCBF in the thalamus [14–16]) and exceeded a threshold of p<0.0001, uncorrected. Changes in visual analogue scale scores across conditions were analyzed for each dimension by using a one-sample two-tailed t test at p<0.05. Pearson correlations were also calculated between any significant changes in visual analogue scale scores and the changes in rCBF between conditions at p<0.05. For this purpose, rCBF values were obtained for the regions identified as showing differences between conditions, including those voxels that showed levels of significance of p<0.0001, uncorrected for multiple comparisons.

**Results**

**Gender Differences**

No significant gender differences were found in age (women: mean=24.7 years, SD=7.8; men: mean=29.8 years, SD=12.4), number of years smoked (women: mean=5.6, SD=4.8; men: mean=7.9, SD=8.0), Fagerström scores (women: mean=3.0, SD=0.3; men: mean=4.5, SD=2.5), arterial nicotine values (women: mean=3.3 ng/ml, SD=2.6; men: mean=2.6 ng/ml, SD=2.3), and arterial cotinine values (women: mean=91.3 ng/ml, SD=62.3; men: mean=110.6 ng/ml, SD=59.5) (unpaired, two-tailed t tests, p>0.05). However, the average number of cigarettes smoked per day was higher in men than in women (men: mean=19.0, SD=6.6; women: mean=13.5, SD=3.0) (t=2.47, df=16, p=0.02). There was no main effect of gender on RCBF responses (unpaired, two-tailed t tests, p>0.05), and therefore the data for men and women were combined for all analyses.

**Visual Analogue Scale Scores**

The only statistically significant change in visual analogue scale scores from before smoking to after smoking was a decrease in the score for craving from before to after the first cigarette (mean=5.1, SD=2.6, to mean=2.8, SD=2.5) (t=2.11, df=16, p<0.01). There was no change in craving from before to after the second average-nicotine cigarette or from before to after the denicotinized cigarette. Furthermore, there were no changes in score for feeling relaxed, sick, wakeful, and nervous from pre- to postsmoking for any cigarette. To determine whether the average-nicotine and denicotinized cigarettes had similar effects on these measures, we conducted an unpaired t test comparing the change in visual analogue scale scores from before to after the second average-nicotine cigarette for those who smoked it second and from before to after the denicotinized cigarette for those

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**References**

http://ajp.psychiatryonline.org/cgi/content/full/162/3/567
who smoked it second. In this way, the previous experience was held constant—i.e., both groups had smoked an average-nicotine cigarette 24 minutes earlier. No significant differences were found, although a difference in scores indicating an increase in sickness after the second average-nicotine cigarette but not after the denicotinized cigarette approached significance \((t=2.03, \text{df}=17, p=0.06)\).

Pharmacokinetic and Cardiovascular Data

Peak plasma levels of nicotine were higher during the second average-nicotine cigarette than during the first (mean=59 ng/ml, SD=38, versus mean=41 ng/ml, SD=21) \((t=2.85, \text{df}=16, p<0.05)\). Given that the half-life of nicotine is greater than 1 hour, this difference was presumably related to carryover effects, because subjects smoked the second average-nicotine cigarette 24 or 48 minutes after the first. However, the increase in peak plasma levels of nicotine from before to after smoking was not significantly different between the first and second average-nicotine cigarettes (mean=40 ng/ml, SD=21, and mean=52 ng/ml, SD=37, respectively) \((t=1.86, \text{df}=15, p=0.08)\). Compared to the peak plasma level of nicotine associated with the denicotinized cigarette, the peak plasma levels of nicotine were much greater for both the first average-nicotine cigarette (mean=16 ng/ml, SD=8, versus mean=43 ng/ml, SD=20) \((t=6.98, \text{df}=16, p<0.001)\) and the second average-nicotine cigarette (16 ng/ml, SD=9, versus mean=61 ng/ml, SD=38) \((t=5.41, \text{df}=15, p<0.001)\). The increase in peak plasma nicotine levels from pre- to postcigarette was also much greater for the first average-nicotine cigarette than for the denicotinized cigarette (mean increase=40 ng/ml, SD=20, versus mean increase=3 ng/ml, SD=3) \((t=7.77, \text{df}=16, p<0.001)\) and for the second average-nicotine cigarette than for the denicotinized cigarettes (mean increase=54 ng/ml, SD=37, versus mean increase=3 ng/ml, SD=3) \((t=5.58, \text{df}=14, p<0.001)\).

Because the half-life of nicotine is 1 hour and the order of the second average-nicotine cigarette and denicotinized cigarette was randomized between subjects, there was some concern that this aspect of the study design may have resulted in a large carryover effect for subjects who smoked the denicotinized cigarette third, given that they had already smoked two average-nicotine cigarettes. For this reason, we looked at the differences in peak nicotine level after the denicotinized cigarette between those who smoked it second and those who smoked it third. We found no significant difference (mean=14 ng/ml, SD=9, versus mean=20 ng/ml, SD=7 ng/ml) \((t=1.71, \text{df}=15, p=0.11)\). There was also no significant difference in peak nicotine level after the second average-nicotine cigarette between those who smoked it second and those who smoked it third (mean=53 ng/ml, SD=33, versus mean=64 ng/ml, SD=44) \((t=0.61, \text{df}=15, p=0.54)\).

There were no significant differences in peak heart rate, diastolic blood pressure, or systolic blood pressure after smoking the first versus the second average-nicotine cigarette. Compared to smoking the denicotinized cigarette, smoking the first average-nicotine cigarette resulted in a significant increase in heart rate (mean=84 bpm, SD=8, versus mean=97 bpm, SD=11) \((t=5.65, \text{df}=16, p<0.05)\) and systolic blood pressure (mean=126, SD=13, versus mean=128, SD=14) \((t=2.32, \text{df}=16, p<0.05)\), but not diastolic blood pressure (mean=67, SD=11, versus mean=68, SD=8) \((t=1.43, \text{df}=16, p=0.17)\), and the second average-nicotine cigarette resulted in a significant increase in heart rate (mean=84 bpm, SD=9, versus mean=95 bpm, SD=10) \((t=7.17, \text{df}=15, p<0.001)\) and systolic blood pressure (mean=126, SD=13, versus mean=129, SD=11) \((t=3.30, \text{df}=15, p<0.01)\), but not diastolic blood pressure (mean=67, SD=11, versus mean=69, SD=11) \((t=2.01, \text{df}=15, p=0.06)\).

**rCBF**

Partial data were lost for three female subjects. Because of computer failures during data acquisition, data from the baseline scan were lost for one subject and data from the scan during the first average-nicotine cigarette were lost for another subject. Data from the scan during the second average-nicotine cigarette were lost for a third subject because of radiotracer synthesis failure. The remaining data for each subject were used for the following analysis.

Significant differences in rCBF from the initial baseline scan to the scan during the first average-nicotine cigarette were observed (Table 1 and Figure 1). Significantly higher rCBF during the first average-nicotine cigarette, compared to the preceding baseline, was observed in the right occipital cortex (Brodmann’s area 17/18) and the cerebellum bilaterally. Significant reductions in rCBF during the first cigarette were observed in the occipital cortex bilaterally (Brodmann’s area 18/19), right parietal cortex (Brodmann’s area 1/2), right fusiform gyrus, and right hippocampus and in a large area that overlapped both the ventral anterior cingulate (Brodmann’s area 25) and the nucleus accumbens bilaterally. When the size of the region involved was taken into account with cluster-level correction for multiple comparisons (30), the left dorsal anterior cingulate (Brodmann’s area 32/24) also showed decreased rCBF during the first cigarette, compared to baseline.

| TABLE 1 |

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Differences in rCBF between the scan during the first cigarette of the day and the scan during the denicotinized cigarette were also tested for statistical significance to determine the effect of smoking the first cigarette of the day, with control for nonnicotine aspects of smoking (Table 2 and Figure 2). Significantly higher rCBF during the first average-nicotine cigarette, compared to the denicotinized cigarette, was observed in the cerebellum bilaterally. With cluster-level correction for multiple comparisons, the bilateral occipital cortex (Brodmann’s area 17/18) also showed increased rCBF. Furthermore, the left thalamus, an area for which we had an a priori hypothesis based on our previous data and those from other groups, registered enhancements in rCBF during the first cigarette, compared to the denicotinized cigarette. These increases in rCBF were slightly below the standard threshold of significance after correction for multiple comparisons (p<0.0001, uncorrected). It is noteworthy that lower rCBF during the first cigarette, compared to the denicotinized cigarette, was observed in the nucleus accumbens bilaterally, the left rostral and ventral anterior cingulate and the adjacent prefrontal cortex (Brodmann’s area 10/25/32), the amygdala bilaterally, and the right hippocampus.

We then determined the effect of subsequent cigarette smoking by comparing rCBF responses during the second average-nicotine cigarette to those during the denicotinized cigarette (randomized order of conditions).
(Table 3 and Figure 3). Significantly higher rCBF during the second average-nicotine cigarette, compared to the denicotinized cigarette, was observed in the left occipital cortex (Brodmann’s area 17/18) and the right inferior parietal lobe (Brodmann’s area 40). When the size of the region involved was taken into account with cluster-level correction for multiple comparisons, the right occipital cortex (Brodmann’s area 17/18), left inferior parietal lobe (Brodmann’s area 40), and posterior cingulate (Brodmann’s area 31/23) also showed increased rCBF. Furthermore, the left thalamus registered higher rCBF responses to the second average-nicotine cigarette, compared to the denicotinized cigarette, that were slightly below the standard threshold of significance after correction for multiple comparisons (p<0.0001, uncorrected). Significant reductions in rCBF during the second cigarette, compared to the denicotinized cigarette, were observed in the left rostral and ventral anterior cingulate and adjacent prefrontal cortex (Brodmann’s area 9/10/25/32) and in the right nucleus accumbens. When the size of the region involved was taken into account with cluster-level correction for multiple comparisons, the right hippocampus also showed decreased rCBF in this analysis.

The differences in rCBF responses (both increases and reductions) between the first and second average-nicotine cigarettes were then tested for statistical significance. The first cigarette of the day induced more prominent rCBF increases in the cerebellum bilaterally. In addition, the first cigarette of the day was associated with more prominent reductions in rCBF in the ventral pallidum bilaterally, extending to the nucleus accumbens, compared to the second cigarette of the day (p<0.0001, uncorrected).

As reported earlier, the only statistically significant change in visual analogue scale scores was a decrease in craving from before to after the first cigarette. To further examine this finding, a correlation analysis was performed between the significant changes in rCBF from baseline to the first cigarette (Table 1) and the change in visual analogue scale craving scores during this time (Pearson’s correlations, p<0.05). The decrease in craving from baseline to after the first cigarette was smoked was correlated with the decrease in rCBF in the left dorsal anterior cingulate (r=0.49, N=17, p<0.05) and the right hippocampus (r=0.50, N=17, p<0.05).

**Discussion**

The current data confirm and expand previous data demonstrating complex and bidirectional changes in brain regional synaptic activity, as measured with PET and rCBF measures, during tobacco smoking. Smoking cigarettes with average nicotine content (1 mg of nicotine per cigarette) resulted in rCBF increases in posterior brain areas, primarily the visual cortex and cerebellum, compared to both baseline and denicotinized cigarette (0.08 mg of nicotine per cigarette) conditions. Conversely, consistent reductions in rCBF were observed in anterior and ventral brain areas.
after subjects smoked cigarettes were observed in anterior limbic and paralimbic regions, compared to both baseline and the denicotinized cigarette condition. Furthermore, the reductions in cigarette craving scores obtained after subjects smoked the first cigarette of the day were correlated with the reductions in rCBF in the anterior cingulate and hippocampus.

Possible differences in smoking topographies between the two average-nicotine cigarettes and between the average-nicotine cigarettes and the denicotinized cigarette were not addressed in a standardized fashion, except for instructing the subjects about the frequency of the puffs and controlling for the total number of puffs per cigarette. However, the visual analogue scale data indicated that the two smoking preparations were similar in their psychological effects. There were no differences in ratings of craving, feelings of being relaxed, sick, wakeful, or nervous between the randomized average-nicotine cigarette and denicotinized cigarette conditions. In addition, the pharmacokinetic and cardiovascular data indicated no significant differences in the effects of the two average-nicotine cigarettes on these measures, suggesting that carryover effects from the first to the second average-nicotine cigarette did not affect the change in plasma levels or physiological responses to nicotine achieved during the two administrations.

Previously published data have conflicted in terms of both the direction of change and the brain regions affected by nicotine and tobacco smoking. Some of the variability in the results may be related to the status of the subjects at the time of the study (e.g., withdrawal status or time since last cigarette), as well as to the brain regions examined and the mode of nicotine administration. Previous studies used intravenous nicotine administration (12, 19), nicotine gum (20), and a nicotine nasal spray (14–16). The results of these studies are difficult to interpret either because of the nontypical routes of nicotine administration used (e.g., intravenous administration) or because the changes in plasma levels do not reflect those obtained with tobacco smoke inhalation (e.g., changes in plasma levels with use of nicotine gum or nasal spray). In the current study, we chose to directly examine the effects of smoking a cigarette with average nicotine content, compared either with a baseline state (no smoking) or with smoking a cigarette with low nicotine content. Furthermore, we were able to investigate separately the effects of the first cigarette of the day, after about 12 hours of abstinence, and a cigarette smoked later in the morning.

rCBF Increases

Increases in rCBF were observed in all comparisons in the primary visual cortex, an area consistent with the known brain distribution of pathways regulated by nicotinic receptors in animals and humans (14, 32, 33). Increases in rCBF in the thalamus, a region rich in nicotinic receptors, were also observed in the comparisons of the effects of the first or second nicotine-containing cigarette with those of the denicotinized cigarette. Although these results are consistent with the results obtained in previous work by our group and others (12, 14–17), the current results reached only subthreshold levels of significance after full correction for multiple comparisons. Increases in cerebellar rCBF were also detected, a finding that is consistent with a number of previous reports (13–15). However, these increases were found only in comparisons involving the first average-nicotine cigarette, compared with the baseline or denicotinized cigarette conditions, and not in comparisons involving the second average-nicotine cigarette. Decreases in rCBF in the cerebellum have been reported while volunteers performed a psychometric task after intravenous nicotine administration (19). The cerebellum has reciprocal connections to the dorsolateral prefrontal cortex, medial frontal cortex (including the anterior cingulate), parietal and superior temporal cortices, and the posterior hypothalamus (34) and has recently been implicated in cognitive, affective, and behavioral functions along with its traditional role in motor functioning (reviewed in reference 35). The differential effects of the first versus subsequent cigarettes on cerebellar functional responses may be related to the more profound emotional and cognitive effects of the first cigarette of the day in nicotine-dependent subjects.

While subjects smoked the second cigarette, increases in rCBF were also observed in the inferior parietal lobule, an area that, along with the thalamus, is part of a network of brain areas involved in attentional modulation (36). It is relevant, therefore, that nicotine has been found to improve performance on attention tasks (37). In a recent study, transdermal nicotine in smokers was found to improve performance of a rapid visual information-processing task that required sustained attention and to increase task-induced brain activation in the parietal cortex, thalamus, and caudate (21). Therefore, the increase in blood flow in the parietal area in the current study may be related to activation of an attentional network by nicotine.

Regional CBF Decreases

Decreases in rCBF related to smoking a nicotine-containing cigarette were consistently found in the anterior cingulate, prefrontal cortex, nucleus accumbens, and right hippocampus. In the comparison of the effects of the first cigarette of the day to those of the denicotinized cigarette, additional significant reductions in rCBF were observed bilaterally in the amygdala. Consistent with these findings, Rose and colleagues (17) recently reported a nicotine-related decrease in rCBF in the ventral striatum, prefrontal cortex, anterior cingulate, and amygdala. Furthermore, these authors found that this reduction was attenuated when the nicotinic antagonist...
mecamylamine was given, suggesting that these effects are the direct result of nicotine.

The anterior cingulate, prefrontal cortex, nucleus accumbens, amygdala, and hippocampus are all areas implicated in the reinforcing properties of drugs of abuse. It has been shown that intravenous nicotine self-administration in rats activates the prefrontal cortex, anterior cingulate, and nucleus accumbens (38). It is believed that the nucleus accumbens is involved in responding to stimuli with motivational significance (39). Inputs to the nucleus accumbens arising from the prefrontal cortex, anterior cingulate, amygdala, and hippocampus further modulate the function of the nucleus accumbens and its outputs to motor relay circuitry (39).

The comparison that addressed the effect of smoking the first cigarette of the day, which controlled for the effects of both the nonnicotine aspects of smoking and other substances found in cigarette smoke (tar, CO, and CO₂), yielded rCBF decreases bilaterally in the amygdala. This finding is consistent with those of other blood flow studies that used cigarette inhalation (17) and nasal nicotine (16). The amygdala is believed to have a role in conditioned stimulus-reward associations (40–42), and studies suggest that it is involved in the ability of drug-associated cues to precipitate relapse (e.g., reference 43).

Craving

The change in craving from baseline to the first cigarette of the day was found to correlate with rCBF in the left dorsal anterior cingulate and the right hippocampus. The anterior cingulate has extensive connections with the amygdala and the nucleus accumbens, and clinical and animal studies implicate this region in the mediation of both craving and relapse to drug-seeking behavior (reviewed in reference 44). Consistent with this finding, the anterior cingulate cortex was activated in an animal model of nicotine relapse after abstinence (38). Using PET, Brody and colleagues (45) found increases in relative glucose metabolism in the anterior cingulate during cigarette craving. Dorsal anterior cingulate activation has also been observed during cue-induced craving in abstinent opiate-dependent subjects (46), and imaging studies of cocaine craving show consistent activation of the anterior cingulate, prefrontal cortex, and amygdala (40–42, 47–50). Up-regulation of µ-opioid receptors, a neurotransmitter system involved in the effects of various drugs of abuse, including nicotine, has also been demonstrated in these areas in cocaine-dependent volunteers, and this up-regulation further correlated with craving for cocaine on withdrawal (51).

The hippocampus is known to participate in associative processes and is central to the process of associating environmental cues with drugs. Nicotine has been shown to influence forms of synaptic plasticity in the hippocampus that are believed to underlie learning and memory (52, 53). This process is believed to lead to the craving and relapse that can occur years after abstinence from nicotine (54). In an fMRI study of cue-induced craving in nicotine-deprived smokers, D'ur and colleagues (55) found increases in blood oxygenation levels in the right hippocampus, as well as in prefrontal and mesolimbic regions, including the ventral tegmental area and the amygdala. The right hippocampus, along with the right amygdala, has also been shown to be activated during cue-induced alcohol craving in abstinent alcohol-dependent men (56).

Although two average-nicotine cigarettes were smoked in the current study, only the first one caused a significant decrease in craving. This difference may be related to a floor effect—i.e., not enough time elapsed between the first and second average-nicotine cigarettes for much craving to develop, and therefore craving scores could not decrease significantly after the second cigarette. The craving scores support this hypothesis. At the start of the study, the average craving score was 5.2, whereas craving scores ranged from 2.2 to 3.4 afterward, suggesting a decrease in craving after the first cigarette that lasted throughout the study. Furthermore, smoking the second average-nicotine cigarette did not produce a significant change in craving, compared to smoking a denicotinized cigarette. This result may also have been related to the floor effect just described. However, it has also been shown that denicotinized cigarettes have effects on craving similar to that of average-nicotine cigarettes (e.g., reference 57), which suggests that the correlations between changes in rCBF and changes in craving from baseline to the first average-nicotine cigarette may not be attributed solely to nicotine and may also be caused by the act of smoking itself.

Summary

Tobacco smoking is the most frequent form of substance abuse, because of the addictive properties of nicotine. Our findings suggest that smoking affects rCBF not only in those areas of the brain rich in nicotinic receptors but also in areas of the brain implicated in the rewarding effects of drugs of abuse. Furthermore, craving for a cigarette in nicotine addicts was found to be correlated with rCBF in the hippocampus, an area involved in associating environmental cues with drugs, and in the dorsal anterior cingulate, an area previously implicated in the mediation of both craving and relapse to drug-seeking behavior.

Footnotes
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References


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