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Effects of Smoking Cessation on Presynaptic Dopamine Function of Addicted Male Smokers

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ABSTRACT

BACKGROUND: There is evidence of abnormal cerebral dopamine transmission in nicotine-dependent smokers, but it is unclear whether dopaminergic abnormalities are due to acute nicotine abuse or whether they persist with abstinence. We addressed this question by conducting longitudinal positron emission tomography (PET) examination of smokers before and after 3 months of abstinence.

METHODS: We obtained baseline 6-[^{18}F]fluoro-*L*-DOPA (FDOPA)-PET scans in 15 nonsmokers and 30 nicotine-dependent smokers, who either smoked as per their usual habit or were in acute withdrawal. All smokers then underwent cessation treatment, and successful abstainers were re-examined by FDOPA-PET after 3 months of abstinence (n = 15). Uptake of FDOPA was analyzed using a steady-state model yielding estimates of the dopamine synthesis capacity (K); the turnover of tracer dopamine formed in living brain (k_{loss}); and the tracer distribution volume (V_d), which is an index of dopamine storage capacity.

RESULTS: Compared with nonsmokers, K was 15% to 20% lower in the caudate nuclei of consuming smokers. Intraindividual comparisons of consumption and long-term abstinence revealed significant increases in K in the right dorsal and left ventral caudate nuclei. Relative to acute withdrawal, V_d significantly decreased in the right ventral and dorsal caudate after prolonged abstinence. Severity of nicotine dependence significantly correlated with dopamine synthesis capacity and dopamine turnover in the bilateral ventral putamen of consuming smokers.

CONCLUSIONS: The results suggest a lower dopamine synthesis capacity in nicotine-dependent smokers that appears to normalize with abstinence. Further investigations are needed to clarify the role of dopamine in nicotine addiction to help develop smoking prevention and cessation treatments.

Keywords: Abstinence, Dopamine, FDOPA, Nicotine dependence, Smoking cessation, Striatum

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Worldwide, nicotine is the most common drug of abuse and contributes to 8.8% of all deaths (1). The reinforcing properties of nicotine and other addictive drugs are generally associated with stimulated dopamine release in the nucleus accumbens (NAc) (2,3). Several D_2 receptor positron emission tomography (PET) studies showed acutely increased dopamine signaling in ventral striatum after nicotine intake (4,5). Distinct from the acute effects of nicotine on dopamine release, dependency may be promoted by preexisting or acquired deficiencies in the subcortical dopamine transmission/reward system. Many PET studies reported lower striatal D_1 and $D_{2/3}$ receptor availability in smokers (6–9), although other studies did not find significant differences (10–12). Likewise, some, but not all, studies reported reductions in dopamine transporters on nigrostriatal terminals of smokers (10,13–15). Furthermore, Busto *et al.* (16) reported attenuation of the *d*-amphetamine-evoked dopamine release using [^{11}C]raclopride-PET in smokers. Thus, striatal dopamine transmission may be reduced in addicted smokers through presynaptic changes. However, two PET studies with the dihydroxyphenylalanine decarboxylase substrate 6-[^{18}F]fluoro-*L*-DOPA (FDOPA) reported inconsistent results (17,18).

Cross-sectional studies on dopamine transmission in tobacco addiction have an important confound: it is uncertain whether dopaminergic abnormalities in smokers are a consequence of nicotine consumption or are an antecedent trait. Individual variations in dopamine-modulated brain circuits are assumed to constitute a potential risk factor for addiction (19). Preclinical PET results in rhesus monkeys showed that low D_2 receptor availability predicts cocaine self-administration (20), suggesting that low dopamine transmission may predispose to drug use. However, cocaine exposure reduced D_2 receptor availability in monkey striatum, which partly recovered during abstinence. The concurrence of preexisting and acquired states of brain dopamine may contribute to the above-noted heterogeneity in PET findings among smokers.

Human prospective longitudinal studies are mandatory to disentangle predispositions and consequences of smoking,

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but these are impossible to conduct in the field of nuclear imaging for radiation safety reasons because it would demand investigations in a large cohort of adolescents before they start the substance intake. A different approach is to focus on long-term abstinence. If alterations normalize with abstinence, as has been reported for nicotinic acetylcholine receptors (21), this may suggest that they were induced by nicotine consumption, rather than reflecting a predisposition.

The aims of the present study were to investigate presynaptic dopamine synthesis capacity in smokers and its changes during long-term abstinence. Furthermore, we aimed to disentangle effects of acute withdrawal and continuous nicotine consumption, as these states are important to differentiate in nicotine-addicted individuals. To this end, we obtained FDOPA-PET scans of habitual smokers either during ongoing smoking or in acute withdrawal and compared these with a nonsmoking control group. However, a primary goal of the present protocol was to acquire a second FDOPA-PET scan of smokers after they had successfully undergone a cessation treatment program and had remained abstinent for at least 3 months. We hypothesized that dopamine synthesis capacity would be lower in the ventral striatum of smokers compared with nonsmokers. Furthermore, we hypothesized that a low synthesis capacity might constitute a trait factor for nicotine dependence and would persist with long-term abstinence and possibly predict treatment success. In addition, we assumed that acute smoking would stimulate dopamine turnover, which should be reduced under acute withdrawal conditions.

METHODS AND MATERIALS

The study was approved by the Ethics Committee of the Medical Faculty of the RWTH Aachen University and German radiation safety authorities in accordance with national law and international standards including the Declaration of Helsinki and Good Clinical Practice. Participants gave written informed consent for participation in the study.

Participants

Study participants included 30 nicotine-dependent smokers (age, 28.4 ± 7.1 years; range, 19–47 years) and 15 nonsmokers (age, 27.9 ± 7.5 years; range, 19–46 years). All participants were male to avoid confounding effects of sex (22) or menstrual cycle phase (23). Only smokers seeking treatment and presenting with a diagnosis of nicotine dependence (DSM-IV) were included. In addition, smokers with low nicotine dependency were excluded (<3 points on the Fagerström Test for Nicotine Dependence [FTND]) (24). Nonsmokers were defined as persons who had smoked <20 cigarettes in their lifetime. All subjects were screened initially during a telephone interview using a checklist of inclusion and exclusion criteria. Individuals who met initial screening criteria underwent an extensive mental and physical state examination by an experienced psychiatrist including a German short version of the Structured Clinical Interview for DSM disorders (25). Blood analyses, electrocardiography and electroencephalography examinations, and a T1-weighted magnetic resonance imaging (MRI) scan (Philips Achieva 1.5-tesla scanner; Philips Healthcare, Best, The Netherlands) were performed to exclude any relevant somatic diseases. In addition, subjects performed a battery of neuropsychological tests. Except for nicotine dependence in smokers, participants had to be free of any history of drug abuse or dependence, free of history of any other mental disorder, and free from the use of centrally acting medications within the preceding 6 months. According to our protocol, smokers underwent a first PET scan before the cessation treatment and a second scan after 3 months of abstinence. Nonsmokers and relapsing smokers were scanned only once because no significant changes in dopamine function could be expected after 3 months (26) that would justify a second exposure to radiation. The initial group of 30 smokers was randomly assigned to two subgroups for the first scan: one group stayed abstinent for at least 6 hours before scanning ["withdrawal" (27)]; the other group smoked as per their usual habit until approximately 30 minutes before the tracer injection ("ongoing consumption"). Both smoking groups were comparable regarding nicotine dependence and the age of first nicotine use (Table 1). Furthermore, the three groups did not differ in age, education, or intelligence as measured by the Culture Fair Intelligence Test (28).

Smoking Cessation

After PET scanning, all smokers underwent a standardized behavioral therapeutic smoking cessation course according to the manual by Batra and Buchkremer (29). The course consisted of weekly group meetings over a period of 6 weeks conducted by a psychologist with additional qualification in smoking cessation therapy. Every participant was asked to determine his individual quit day between the second and third meeting and to stop smoking completely on that day. Participants were allowed to use nicotine replacement drugs (free of choice), but no other medication was permitted. The time period to follow-up scanning was extended in these cases so that the subjects were free of nicotine in all forms for 3 months. Five subjects used nicotine gum, one used nicotine patches, and another used gum and patches. For abstinence control and as a motivational tool, breath carbon monoxide was measured every week (Breath CO Monitor BMC-2000, Senko Co., Ltd., Gyeonggi-do, Korea) by the smoking cessation therapist. On completion of the group meetings, participants were interviewed about relapses every 2–4 weeks. Participants who reported abstaining from cigarettes as well as nicotine replacement for at least 3 months were invited for follow-up scanning. Blood and urine samples were taken to determine cotinine levels for verification of abstinence (serum cotinine <15 ng/mL and urine cotinine <20 μg/g creatinine).

PET Data Acquisition

The PET scans were performed with a Siemens ECAT HR+ whole-body PET scanner in the three-dimensional mode (Siemens Healthcare, Erlangen, Germany). All participants were asked to refrain from alcohol for 2 days and from food containing high amounts of protein for 1 day before the scan. Decarboxylation of FDOPA in peripheral tissues was reduced by oral administration of 2 mg/kg body weight carbidopa (Lodosyn; Merck & Co., Inc., Whitehouse Station, New Jersey). The dose was administered at two time points: two thirds 1 hour before the scan and one third directly before FDOPA administration. We acquired a sequence of 30 emission frame scans lasting 124 minutes as reported previously (30). The FDOPA (230 ± 12 MBq) was injected as a slow intravenous

Table 1. Demographic Characteristics of Participants

	Ongoing Consumption (n = 15)[a]		Acute Withdrawal (n = 15)[b]		Nonsmokers (n = 15)		
	Mean	SD	Mean	SD	Mean	SD	Group Comparison (p)[c]
Age, Years	27.4	6.5	29.4	7.7	27.9	7.5	.77
Years of Education	15.4	2.0	15.5	2.1	15.4	1.8	.99
IQ (CFT)	111.1	16.9	111.3	15.2	115.0	16.4	.76
FTND Score	5.1	1.6	4.7	1.4	—	—	.56
Cigarettes per Day	18.0	6.1	17.3	6.1	—	—	.77
Age at First Cigarette, Years	15.9	1.5	17.5	3.9	—	—	.31

CFT, Culture Fair Intelligence Test; FTND, Fagerström Test for Nicotine Dependence.

[a]Smokers after nicotine intake.

[b]Smokers being nicotine free for at least 6 hours.

[c]Group differences were assessed using analysis of variance and Student t test or, in cases of not normally distributed data, Kruskal-Wallis test and Mann-Whitney U test.

bolus into a cubital vein. There were no differences in injected activity between the study groups. During PET scanning, we drew arterial blood samples via a catheter placed in a radial artery to measure the plasma radioactivity curve and to detect FDOPA and 3-*O*-methyl-[^{18}F]fluorodopa fractions (Supplement 1).

Image Analysis

All calculations were performed using PMOD version 3.2 (PMOD Technologies Ltd., Zürich, Switzerland). Framewise motion correction and normalization were performed as reported previously (30). For two participants with contraindications against MRI tomography and one participant who stopped his MRI scan prematurely, PET images were normalized to a normalized FDOPA template. The volumes of interest were delineated on a MRI template and were slightly manually adapted, if necessary, on the individual normalized MRI scan. These analytical steps were done by analysts who were blind to subject and conditions. Time activity curves for the cerebellum, the NAc, and dorsal and ventral caudate nucleus (CN) and ventral and dorsal putamen were calculated.

FDOPA Kinetics

The kinetic analysis of the FDOPA time activity curves was performed by application of the reversible inlet-outlet model as described previously (31,32). This model defines a triad of kinetic parameters: 1) the intrinsic blood-brain clearance of FDOPA (K [mL/g/min]), which is an index of dopamine synthesis capacity; 2) the washout rate for [^{18}F]fluorodopamine (k_{loss} [min^{-1}]), which is a surrogate parameter of dopamine turnover; and 3) the steady-state distribution volume of FDOPA together with its decarboxylated metabolites (V_d [mL/g], which is an index of dopamine storage capacity, comparable to the effective distribution volume (mL/g; V_d = Effective distribution volume + V_f + V_0), as defined by Sossi *et al.* (33). The three inlet-outlet model parameters of interest were calculated for 10 volumes of interest: right and left NAc, ventral and dorsal CN, and ventral and dorsal putamen according to the approach of Schlüter *et al.* (30).

Statistical Analyses

The PET data were analyzed using two methods. First, mixed models were fitted with SAS software (SAS Corp., Cary, North Carolina). These models were chosen because they can best represent the hierarchical and correlational structure of the data. The imaging outcome parameters were highly intercorrelated between the brain regions (mean correlation of .65 for K, .58 for V_d, and .48 for k_{loss}). To analyze baseline group differences, models were fitted with fixed factors "group" ("nonsmokers"/"consumption"/"withdrawal"), "age," "brain region," and "hemisphere" as well as "region × hemisphere" and random effects of individual subjects. To investigate the effect of abstinence, additional exploratory models were fitted, which included "time" and "time × group" as additional fixed factors. Second, conventional group differences were assessed using Student t test in IBM SPSS Statistics version 22 (IBM Corp., Armonk, New York). To control for multiple testing, we applied the Dubey/Armitage-Parmar correction (34), which takes into account the degree of correlation among the dependent variables. The significance level was set at $p_{adj} < .05$, two-sided. For intraindividual differences between baseline and long-term abstinence, t tests for paired samples were used. For correlational analyses of PET parameters and FTND scores, length of smoking history, or neuropsychological data, rank-order correlations by Spearman were applied. The significance level for these tests was $p < .05$ (two-sided), and results have to be regarded as exploratory.

RESULTS

Of 30 smokers, 17 stayed nicotine free for >3 months; 2 dropped out because of unexpected medical conditions or relocation, leaving 15 participants who underwent follow-up PET scanning. The first FDOPA-PET scan was performed under ongoing consumption conditions in 7 of the 15 abstinent smokers and under acute withdrawal conditions in 8. Two subjects who underwent follow-up PET scanning had initially used nicotine gum, one for <1 week and the other for 3.5 weeks. The time period to follow-up scanning was extended in these cases (see earlier).

Interindividual Differences Between Smokers and Nonsmokers

Dopamine Synthesis Capacity. For explaining the variance of regional FDOPA K, a mixed model was applied. For factor "group," significant effects of K were observed ($F_{2,426}$ = 15.10, $p < .0001$) (Supplement 1). Post hoc t tests revealed a 15%–20% difference in the CN of smokers during ongoing

Table 2. Regional Dopamine Synthesis Capacity According to the Reversible Inlet-Outlet Model (K [mL/g/min])

	Ongoing Consumption[a]		Acute Withdrawal[b]		Nonsmokers	
	Mean	SD	Mean	SD	Mean	SD
Right Ventral CN	.0157	.0033	.0179	.0041	.0185	.0032
Left Ventral CN	.0148	.0035	.0178	.0042	.0184	.0033
Right Dorsal CN	.0138	.0031	.0160[c]	.0044	.0166	.0031
Left Dorsal CN	.0138[c]	.0026	.0156[c]	.0047	.0157	.0033
Right NAc	.0161[c]	.0046	.0187[d]	.0039	.0187	.0042
Left NAc	.0181[c]	.0033	.0180[e]	.0040	.0190	.0042
Right Ventral Putamen	.0210	.0058	.0213	.0035	.0217	.0046
Left Ventral Putamen	.0212	.0057	.0209	.0040	.0218	.0037
Right Dorsal Putamen	.0210	.0063	.0212	.0040	.0219	.0044
Left Dorsal Putamen	.0214	.0064	.0204	.0041	.0206	.0060

Values indicate the mean and SD of 15 determinations except where otherwise indicated by footnotes (in these cases, regions of single subjects were excluded from consideration because of too much noise in the data).

CN, caudate nucleus; NAc, nucleus accumbens.

[a]Smokers after nicotine intake.

[b]Smokers being nicotine free for at least 6 hours.

[c]$n = 14$.

[d]$n = 12$.

[e]$n = 13$.

consumption versus nonsmokers: K values were significantly lower in the left ventral CN ($t_{28} = 2.961$, $p = .006$, $p_{adj} = .014$, 95% confidence interval [CI] [.001, .006]) and right dorsal CN ($t_{28} = 2.429$, $p = .022$, $p_{adj} = .049$, 95% CI [.0004, .005]) (Table 2 and Figure 1) and not surviving correction for multiple comparisons in the right ventral CN ($t_{28} = 2.310$, $p = .028$, $p_{adj} = .064$, 95% CI [.0003, .005]). Furthermore, we found lower K values among consuming smokers compared with smokers in the acute withdrawal condition in the left ventral CN, but this effect also failed to survive correction for multiple testing ($t_{28} = 2.138$, $p = .041$, $p_{adj} = .092$, 95% CI [.0001, .006]). Regional K of smokers in acute withdrawal and after long-term abstinence did not differ from regional K of nonsmokers (Figure 1).

Dopamine Turnover and Storage Capacity. The mixed models showed no significant group differences in V_d ($p = .512$) or k_{loss} ($p = .473$) between smoking groups at the first scan and nonsmokers. However, a 22% difference in V_d among smokers after long-term abstinence compared with nonsmokers was found in the right NAc ($t_{23.462} = 2.370$, $p = .026$, $p_{adj} = .066$, 95% CI [.163, 2.379]).

Intraindividual Differences Associated With Long-Term Abstinence

Dopamine Synthesis Capacity. The mixed model yielded no general effect of "time" or "time × group" on K values. However, *t* tests for the striatal subregions revealed significant increases in the right dorsal CN (+13%; $t_6 = -2.782$, $p = .032$, 95% CI [−.003, −.0002]) and left ventral CN (+11%; $t_5 = -2.748$, $p = .040$, 95% CI [−.004, −.0001]) and a nearly significant increase in the left NAc (+15%; $t_6 = -2.424$, $p = .052$, 95% CI [−.005, −.00002]) in former smokers who had consumed tobacco at baseline scanning (Figure 1 and Table 3). For the withdrawal group, no significant changes of K values were seen.

Dopamine Turnover and Storage Capacity. The mixed models revealed significant effects of "time × group" for V_d ($p < .0001$) and k_{loss} ($p < .0001$). The *t* tests showed that V_d values in the withdrawal group significantly decreased in the right ventral CN (−31%; $t_7 = 2.778$, $p = .027$, 95% CI [.336, 4.179]) and right dorsal CN (−33%; $t_6 = 3.521$, $p = .013$, 95% CI [.603, 3.348]) (Figure 2), and k_{loss} values significantly increased in the right NAc (+74%; $t_4 = -3.178$, $p = .034$, 95% CI [−.004, −.0003]) and left NAc (+38%; $t_6 = -3.166$, $p = .019$, 95% CI [−.003, −.0003]) after abstinence. Furthermore, results were close to significance for V_d in the right NAc (−48%; $p = .054$), left NAc (−22%; $p = .091$), and left dorsal CN (−34%; $p = .066$) and for k_{loss} in the right ventral CN (+34%; $p = .088$). In contrast, in the consumption group, an increase of V_d was found in the right dorsal putamen (+16%; $t_6 = -2.803$, $p = .031$, 95% CI [−1.710, −.116]).

Behavioral Correlates of PET Parameters

Among consuming smokers ($n = 15$), FTND scores significantly correlated with K and k_{loss} in the right ventral putamen (K, $r = .59$, $p = .020$, 95% CI [.11, .85]; k_{loss}, $r = .62$, $p = .013$, 95% CI [.16, .86]) and left ventral putamen (K, $r = .57$,

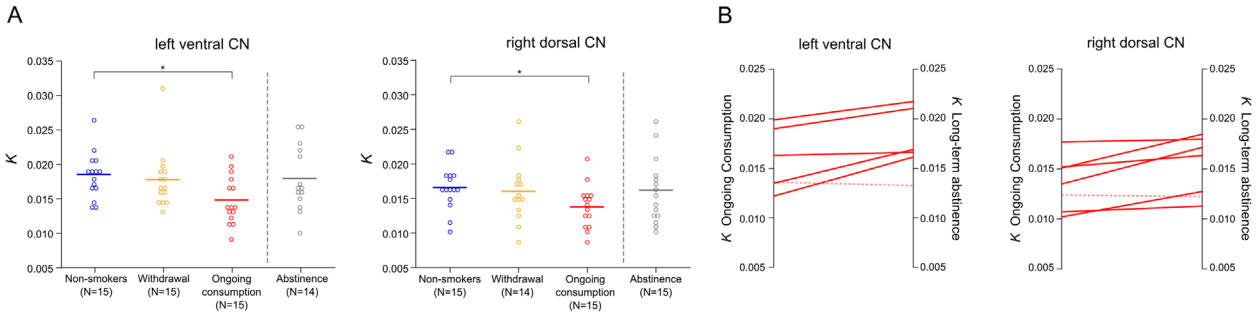

Figure 1. **(A)** Interindividual group differences in dopamine synthesis capacity ([^{18}F]fluoro-*L*-DOPA K) in the left ventral and right dorsal caudate nucleus (CN). *$p < .05$. **(B)** In the same regions, intraindividual changes were found in a group of successfully abstinent smokers relative to a state of ongoing consumption (left ventral CN [$t_5 = -2.748$, $p = .040$, $n = 6$; one subject had to be excluded because of too much noise in the data of the second measurement] and right dorsal CN [$t_6 = -2.782$, $p = .032$, $n = 7$]). Solid lines indicate individual increases, and dashed lines indicate individual decreases.

Table 3. Intraindividual Changes in Regional Dopamine Synthesis Capacity According to the Reversible Inlet-Outlet Model (K [mL/g/min])

	Ongoing Consumption[a]			Acute Withrawal[b]		
	Mean	SD	*p*	Mean	SD	*p*
Right Ventral CN						
First scan	.0155	.0034		.0188	.0047	
Second scan	.0172	.0028		.0183	.0067	
Difference	.0016	.0031	.214	−.0005	.0057	.812
Left Ventral CN						
First scan	.0158[c]	.0032		.0190	.0053	
Second scan	.0176[c]	.0032		.0181	.0058	
Difference	.0019[c]	.0017	.040	−.0008	.0048	.639
Right Dorsal CN						
First scan	.0135	.0027		.0176[d]	.0049	
Second scan	.0152	.0030		.0173[d]	.0064	
Difference	.0016	.0015	.032	−.0003[d]	.0034	.838
Left Dorsal CN						
First scan	.0139	.0031		.0167	.0060	
Second scan	.0155	.0033		.0157	.0049	
Difference	.0016	.0024	.124	−.0010	.0059	.631
Right NAc						
First scan	.0164	.0024		.0160[e]	.0029	
Second scan	.0173	.0026		.0209[e]	.0048	
Difference	.0008	.0033	.526	.0050[e]	.0055	.113
Left NAc						
First scan	.0172	.0039		.0186[d]	.0051	
Second scan	.0197	.0031		.0194[d]	.0045	
Difference	.0025	.0027	.052	.0008[d]	.0028	.477
Right Ventral Putamen						
First scan	.0220	.0068		.0221	.0036	
Second scan	.0198	.0018		.0218	.0047	
Difference	−.0022	.0054	.321	−.0003	.0040	.836
Left Ventral Putamen						
First scan	.0210	.0069		.0217	.0045	
Second scan	.0194	.0020		.0223	.0063	
Difference	−.0017	.0067	.535	.0007	.0053	.734
Right Dorsal Putamen						
First scan	.0226	.0077		.0227	.0039	
Second scan	.0207	.0021		.0219	.0052	
Difference	−.0019	.0072	.510	−.0008	.0038	.554
Left Dorsal Putamen						
First scan	.0220	.0079		.0214	.0040	
Second scan	.0207	.0025		.0221	.0056	
Difference	−.0012	.0068	.645	.0008	.0054	.703

Values indicate the mean and SD of 7 determinations for the consumption group and 8 determinations for the withdrawal group except where otherwise indicated by footnotes (in these cases, regions of single subjects were excluded from consideration because of too much noise in the data).

CN, caudate nucleus; NAc, nucleus accumbens.

[a]Smokers after nicotine intake at the first scan.

[b]Smokers being nicotine free for at least 6 hours at the first scan.

[c]$n = 6$.

[d]$n = 7$.

[e]$n = 5$.

$p = .023$, 95% CI [.08, .84]; k_{loss}, $r = .65$, $p = .008$, 95% CI [.21, .87]). In the acute withdrawal group, PET parameters did not correlate with FTND scores. For correlational analyses of PET parameters and length of smoking history and neuropsychological data and for analyses on the prediction of treatment success see Supplement 1.

DISCUSSION

The present PET study focused on presynaptic dopamine function in nicotine-addicted subjects before and after several months of smoking cessation. Our main finding was a 15%–20% difference in dopamine synthesis capacity (FDOPA K) in the CN of ongoing consuming smokers compared with nonsmokers or smokers in acute withdrawal, a difference that completely normalized with abstinence. Follow-up scans performed 3 months after smoking cessation revealed that the initially lower K values significantly increased in the left ventral and right dorsal CN and became similar to that of nonsmokers in all regions of interest. The other parameters of the FDOPA inlet-outlet model triad—dopamine turnover rate (k_{loss}) and storage capacity (V_d)—showed no significant differences between smokers during ongoing consumption or acute withdrawal and nonsmokers. However, we found decreased V_d in the right NAc in abstinent smokers compared with nonsmokers. Within intraindividual comparisons, V_d was found to decrease significantly in the CN and k_{loss} was found to increase in the NAc from withdrawal to abstinence conditions.

The finding of lower dopamine synthesis capacity in smokers is in agreement with our hypothesis and parallels previous molecular imaging findings of lower availability of presynaptic structures of the dopamine transmission system (primarily dopamine transporters) (10,13,14,16) as well as observations of decreased dopamine metabolites in cerebrospinal fluid of current smokers (35). Our main outcome parameter is synthesis capacity, which does not relate in a simple way to the phasic dopamine release thought to be increased by nicotine and other substances of abuse (4,36,37). Our investigation was not designed as a pharmacologic challenge; rather, it aimed to include addicted subjects who were smoking as per their usual habit up to the time of PET scanning. The dopamine turnover surrogate parameter k_{loss} was not increased in this group relative to nonsmokers, in line with findings showing that although drugs of abuse enhance phasic dopamine release, they attenuate tonic dopamine levels after chronic exposure (38). For example, a study of single unit scans in rats in vivo revealed that nicotine administration favors phasic burst firing but depresses tonic dopamine release in the NAc and in that way increases the signal-to-noise relationship of dopamine transmission (39). This tonic/phasic shift may result in aberrant goal-directed behaviors promoting drug seeking and the development of addiction (38).

Findings of two previous FDOPA-PET studies in smokers are in contrast to the present results. Bloomfield *et al.* (18) did not find a difference in striatal FDOPA use between smokers and nonsmokers. However, their study differed in several important respects from the present investigation: not all smokers met DSM-IV criteria for nicotine dependence, FTND scores were not assessed, and mean cigarette consumption

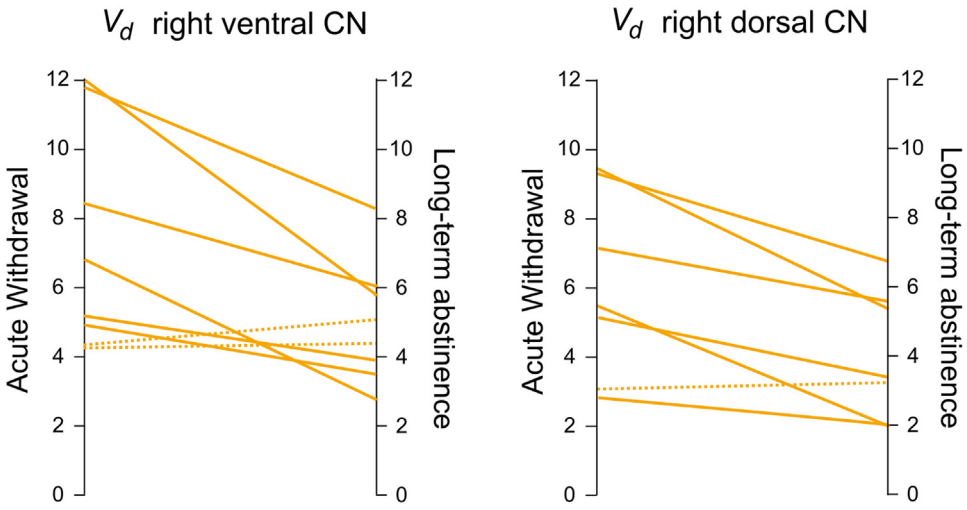

Figure 2. Significant intraindividual changes in dopamine storage capacity ([^{18}F]fluoro-*L*-DOPA V_d) in the right caudate nucleus (CN) (ventral [t_7 = 2.778, p = .027, n = 8] and dorsal [t_6 = 3.521, p = .013, n = 7]) in successfully abstinent smokers, relative to a state of acute withdrawal. Solid lines indicate individual decreases, and dashed lines indicate individual increases.

per day was less than half that of the present sample. Furthermore, smokers abstained from tobacco for 3 hours before the scan, which may have elicited withdrawal symptoms. We found no evidence of differing synthesis capacity between smokers in acute withdrawal and nonsmokers. The finding of increased synthesis capacity in smokers by Salokangas *et al.* (17) is more difficult to explain. However, a close consideration of their results shows that FDOPA kinetics in most striatal subregions did not differ significantly between smokers and nonsmokers, and the reported differences might be explicable by an outlier. Furthermore, smokers abstained for 2–3 hours before the scan; thus, they were in a condition lying between acute consumption and withdrawal.

Our second main hypothesis needs to be clearly rejected: the detected alterations in synthesis capacity (K) in the CN did not persist after long-term abstinence. Despite the small sample size of the subgroup for follow-up analysis, this finding is most likely not an issue of low statistical power; the K values of abstinent smokers were almost identical to the K values of the control group, and intraindividual contrasts revealed significant increases. Thus, there is no evidence for an impaired dopamine synthesis capacity as a marker for vulnerability to nicotine addiction or long-term effects of smoking on dopamine function. The data rather suggest that K is lower than in nonsmokers as a consequence of chronic smoking and normalizes through prolonged abstinence, in analogy to the previously reported changes in D_1 receptors (9), metabotropic glutamate receptors (40), and nicotinic acetylcholine receptors, which were found to normalize to nonsmoker levels by 6–12 weeks of abstinence (21). A biasing effect of treatment success might account for this observation. Smokers who succeed in quitting with a behavioral intervention might possess less severe biological vulnerability as suggested by research on cocaine or stimulants (41,42). Thus, it is possible that a more severe trait-like dopamine deficit might not reverse during abstinence, but that smokers with this deficit are less represented in follow-up comparisons. The PET parameters in the group of ongoing consuming smokers did not predict treatment success. However, among smokers in acute withdrawal, K values in the right NAc predicted treatment success. This result has to be carefully regarded as exploratory but might support the assumption of a biasing effect of treatment success. However, a longitudinal FDOPA study on chronic amphetamine exposure in vervet monkeys also hinted at a recovery of presynaptic dopamine function with abstinence (43), and dopamine transporter levels were found to recover during abstinence from various drugs of abuse (44).

In an exploratory analysis, the magnitudes of dopamine synthesis capacity (FDOPA K) and storage capacity (FDOPA k_{loss}) correlated significantly with FTND scores of nicotine dependence in the bilateral ventral putamen of consuming smokers. This association was absent in the ventral CN of smokers where the group differences in K were seen. The correlations seem reminiscent of earlier findings of a relationship between FTND scores or desire to smoke and $D_{2/3}$ receptor binding in the putamen that was absent or less distinct in the caudate (7,37). The effects of nicotine on dopamine transmission are known to be region specific as a result of distinct expressions of nicotinic acetylcholine receptor subtypes in striatal subregions (45,46). Furthermore, heavier lifetime smoking was found to be associated with smaller NAc volumes but larger left putamen volumes (47). There is evidence that the dorsal striatum plays a key role in the transition from voluntary to habitual drug use (48). Further investigation is needed to disentangle the specific roles of the striatal subregions in nicotine addiction.

The dopamine synthesis capacity (K) of smokers in withdrawal did not differ from that in nonsmokers and was trendwise higher in the left ventral CN compared with consuming smokers. At first glance, this finding may give the impression that dopamine function normalizes within a few hours. However, the changes in striatal storage capacity (V_d) and dopamine turnover (k_{loss}) do not point toward a normalization (Supplement 1): our data suggest intraindividual withdrawal-associated reductions of k_{loss} (significant in bilateral NAc). Thus, higher K values might be explained by feedback mechanisms caused by acute withdrawal. Because of dosimetry issues forbidding three scans per individual, we could not test for intraindividual comparisons between present consumption and withdrawal. Nevertheless, previous studies support our observation that provoked states of withdrawal

caused reduced levels of extracellular dopamine in rodent NAc (49–52). An increase of K after several hours of nonsmoking might be counter-regulative under the influence of reduced dopamine transmission in the withdrawal state. Striatal dopamine synthesis capacity is a matter of regulation under antidopaminergic conditions (53). We previously reported stimulation of striatal FDOPA uptake after $D_{2/3}$ receptor antagonistic challenge that was most likely due to inhibition of presynaptic autoreceptors (54), suggesting that acute withdrawal may result in feedback activation of dopamine synthesis. Further research is needed to elucidate these processes and to infer implications for cessation treatment. For example, the alterations of presynaptic dopamine function found in this study may contribute to the high risk of relapse during the beginning of abstinence. We reported elsewhere that the magnitude of k_{loss} in the left ventral striatum of detoxified alcoholics correlates with craving (55), which is a strong predictor of relapse.

In contrast to results for dopamine synthesis capacity, storage capacity (V_d) differs between abstinent smokers and nonsmokers in the right NAc, paralleling findings of lower striatal V_d in detoxified alcoholics compared with healthy control subjects (55). We did not find group differences under smoking conditions (baseline). Future studies are needed to investigate whether low storage capacities might reflect a vulnerability factor.

The present study has some limitations. First, results of the intraindividual comparisons must be regarded as exploratory because group sizes are very small. Thus, statistical power is limited, and several contrasts are only close to statistical significance despite very high percentage differences. Even the significant findings would not survive correction methods for multiple testing, in contrast to interindividual group comparisons. As noted earlier, we were limited to two scans per individual, which lessened the sensitivity for detecting differences between ongoing consumption and withdrawal. Second, an inclusion bias may be present because our subjects had high education, were free of any psychiatric disorder, did not abuse substances other than nicotine, sought treatment, and had moderate FTND scores; this might limit the generalizability of the present findings. Third, FDOPA metabolism in the brain is very complex, and the inlet-outlet model for steady-state analysis is relatively new. However, previous studies on disturbed dopamine function in schizophrenia (56) and the effects of methylphenidate on striatal dopamine turnover (53) confirm both the very good validity and the relevance of the inlet-outlet model as a tool indicating presynaptic changes in the nigrostriatal dopamine system. Finally, only male subjects were included in the study. Sex differences in addictive disorders have been reported repeatedly—for example, regarding vulnerability and prevalence or the biological responses to drugs (57,58). A study using FDOPA in healthy nonsmokers revealed sex differences in presynaptic striatal dopamine function (59). Further studies are needed that investigate whether such differences in the dopamine system might be associated with liability to nicotine dependence.

In conclusion, the present investigation is the first study on presynaptic dopamine function that examined smokers before and several months after abstinence. By focusing on the effects of smoking cessation, it provides new insights into dopamine transmission in nicotine-dependent smokers. The results suggest a lower dopamine synthesis capacity in smokers that appears to normalize with abstinence. Thus, a marked impairment after cessation reflecting a possible trait for addiction could not be found. These findings suggest that a reduced sensitivity in the dopamine system might rather be a consequence of chronic smoking. Future research is needed to determine whether an impaired dopamine system also constitutes a source of vulnerability to nicotine addiction and whether the results of the present investigation can be generalized to other drugs of abuse.

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