



# Enhanced dopamine release by nicotine in cigarette smokers: a double-blind, randomized, placebo-controlled pilot study

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## Abstract

Previous studies of smoking on dopamine release in humans were investigated only in smokers. Using nicotine gum, we examined the effect of nicotine on dopamine release in smokers and non-smokers and its relation to the degree of nicotine dependence. Smokers and non-smokers participated in a double-blind, randomized, placebo-controlled cross-over study. They participated in two PET measurements with [<sup>11</sup>C]raclopride, in which they received either nicotine or placebo. Changes in [<sup>11</sup>C]raclopride non-displaceable binding potential (BP<sub>ND</sub>) following nicotine administration were quantified. Smokers showed significant decrease in BP in the striatum following nicotine administration, but non-smokers did not show such a decrease. The BP<sub>ND</sub> difference between the two scanning sessions was correlated with the degree of nicotine dependence. The BP<sub>ND</sub> difference might reflect enhanced dopamine release in smokers and the reinforced effect of nicotine. These data suggest the feasibility of our gum method as well as the importance of the degree of dependence in future studies of the nicotine effect on the dopamine system.

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## Introduction

Nicotine is a major psychostimulant component of tobacco. Repeated nicotine exposure can induce nicotine dependence (Laviolette and van der Kooy, 2004; Olsson et al., 2003). It has been suggested that the mesolimbic dopamine pathway is involved in nicotine dependence (Yasuno et al., 2007). [<sup>11</sup>C]raclopride has been used for the indirect measurement of changes in synaptic dopamine concentration in vivo using PET in response to addictive drugs like cocaine and amphetamine (Dewey et al., 1993). Dopamine is thought to compete with [<sup>11</sup>C]raclopride at the D<sub>2</sub> receptor, and dopamine release is associated with

a reduction in [<sup>11</sup>C]raclopride binding (Dewey et al., 1993). Decreases in [<sup>11</sup>C]raclopride binding potential (BP) in the ventral striatum have been demonstrated in smokers following cigarette smoking (Brody et al., 2004, 2006; Scott et al., 2007). On the other hand, two human PET studies of smokers (Barrett et al., 2004; Montgomery et al., 2007) and an awake-monkey study (Tsukada et al., 2002) showed no overall changes in [<sup>11</sup>C]raclopride BP after exposure to nicotine. However, the monkeys were nicotine-naïve, and the study by Montgomery et al. mainly examined low-dependence smokers. It can be expected that the degree of nicotine dependence affects dopamine release in the brain (Scott et al., 2007). In this study, we used nicotine gum with the aim of exposing non-smokers to nicotine to the same degree as smokers. Another objective of this pilot study was to examine the feasibility of nicotine gum methods. The study was conducted in a double-blind, randomized, placebo-controlled manner.

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## Method

### Participants

Twelve male subjects (six smokers, mean age  $25.8 \pm 2.6$  yr, and six non-smokers,  $23.7 \pm 2.7$  yr) participated in a double-blind, randomized, placebo-controlled, cross-over pilot study. Smokers had a smoking history of at least 4 yr, with current use of  $\geq 15$  cigarettes per day. The Fagerstrom test for nicotine dependence (FTND) was applied (Heatherton et al., 1991). The FTND, consisting of six questions (e.g. How soon after you wake up do you smoke your first cigarette? How many cigarettes per day do you smoke?), yields a score ranging from 0 to 10 (0–2, very low dependence; 8–10 very high dependence). The non-smokers had no history of recreational use of cigarettes. None of the subjects were taking alcohol at the time, nor did they have a history of psychiatric disorder, significant physical illness, head injury, neurological disorder, or alcohol or drug (other than nicotine) dependence. MRI demonstrated intact cerebral structures in all subjects. All subjects were right-handed according to the Edinburgh Handedness Inventory. Smokers were instructed not to smoke for 24 h before scanning, and abstinence was verified by plasma nicotine measurement. Both before and after the administration of nicotine, the strength of cigarette craving was assessed using a 6-point scale (0 = no urge, 5 = extremely strong urge). After description of the study to the subjects, written informed consent was obtained, and the study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Japan.

### Nicotine administration

Each subject participated in two PET sessions. To ensure maximum and stable plasma concentrations of nicotine during the PET scans, 1 h before each scan the subjects received two pieces of either nicotine (2 mg Nicorette, mint taste; Pfizer, Tokyo, Japan) or taste-matched placebo gum. A clinical research coordinator (Y.F.), generated the randomization sequence (the order of the two sessions) and packaged the placebo and nicotine gum in containers according to the balanced randomization list (half of the subjects took nicotine gum first, and the remaining half took placebo gum first). The participants and all study staff and investigators, except Y.F., remained blinded to the treatment allocation throughout the study. Every 3 min, the subjects chewed the gum five times at a rate of 1 Hz and then put the gum into the oral vestibule in front of the lower anterior teeth. Until the start of the PET

scans, the subjects were trained to chew the gum while not moving the maxilla but moving only the mandible in order to minimize head motion associated with jaw motion during mastication. The participants kept chewing the gum in the same way during the scans, and finally finished chewing at the end of the scans. Blood samples for measurement of plasma nicotine concentration were collected just before gum administration, and at 60 min, 75 min, 90 min, 105 min, and 120 min after gum administration.

### PET scan

PET studies were performed on ECAT EXACT HR+ (CTI-Siemens, Knoxville, TN, USA). The system provides 63 planes and a 15.5-cm field of view. To minimize head movement, a head fixation device (Fixster, Stockholm, Sweden) was used. A transmission scan for attenuation correction was performed using a germanium-68–gallium-68 source. Acquisitions were performed in 3D mode with the interplane septa retracted. A bolus of  $225.1 \pm 9.7$  MBq of [ $^{11}\text{C}$ ]raclopride with a specific radioactivity of  $262.0 \pm 97.6$  GBq/ $\mu\text{mol}$  was injected intravenously from the antecubital vein with a 20-ml saline flush. Dynamic scans were performed for 60 min immediately after the injection. All emission scans were reconstructed with a Hanning filter cut-off frequency of 0.4 (full width at half maximum, 7.5 mm). MRI was performed on Gyroscan NT (Philips Medical Systems, Best, The Netherlands) (1.5 T). T1-weighted brain images were obtained for all subjects. The scan parameters were 1-mm-thick, 3D T1 images with a transverse plane (repetition time/echo time, 19/10 ms; flip angle,  $30^\circ$ ; scan matrix,  $256 \times 256$  pixels; field of view,  $256 \times 256$  mm; number of excitations, 1).

### Data analysis

The tissue concentration of radioactivity was obtained from volumes of interest (VOIs) defined on PET images with reference to the individual MRIs co-registered on summated PET images and a brain atlas. The regions were the right and left dorsal caudate, dorsal putamen, ventral caudate, and ventral putamen. Each VOI consisted of three slices. The dorsal boundary of the dorsal caudate was at the level of the interventricular foramen of Monro. The dorsal boundary of the dorsal putamen was two slices lower than that of the dorsal caudate. The ventral boundary of the ventral caudate was at the level of the lower boundary of the third ventricle. The ventral boundary of the ventral putamen was one slice higher than that of the ventral caudate. Quantitative analysis was

**Table 1.** [ $^{11}\text{C}$ ]raclopride  $\text{BP}_{\text{ND}}$  (mean  $\pm$  s.d.) in the striatal regions of smokers and non-smokers

	Smokers		Non-smokers	
	Placebo	Nicotine	Placebo	Nicotine
Right dorsal caudate	3.00 $\pm$ 0.16	2.87 $\pm$ 0.26	2.89 $\pm$ 0.48	2.93 $\pm$ 0.30
Left dorsal caudate	3.02 $\pm$ 0.22	2.85 $\pm$ 0.33	2.84 $\pm$ 0.36	2.93 $\pm$ 0.28
Right dorsal putamen	3.77 $\pm$ 0.33	3.52 $\pm$ 0.47	3.67 $\pm$ 0.39	3.62 $\pm$ 0.24
Left dorsal putamen	3.72 $\pm$ 0.39	3.50 $\pm$ 0.43	3.59 $\pm$ 0.42	3.65 $\pm$ 0.23
Right ventral caudate	2.74 $\pm$ 0.24	2.44 $\pm$ 0.18	2.47 $\pm$ 0.27	2.55 $\pm$ 0.29
Left ventral caudate	2.77 $\pm$ 0.26	2.52 $\pm$ 0.22	2.56 $\pm$ 0.36	2.62 $\pm$ 0.25
Right ventral putamen	3.66 $\pm$ 0.25	3.31 $\pm$ 0.21	3.27 $\pm$ 0.39	3.35 $\pm$ 0.32
Left ventral putamen	3.53 $\pm$ 0.40	3.30 $\pm$ 0.25	3.33 $\pm$ 0.43	3.41 $\pm$ 0.25
Striatal region <sup>a</sup>	3.28 $\pm$ 0.32	3.04 $\pm$ 0.24	3.08 $\pm$ 0.32	3.13 $\pm$ 0.24

$\text{BP}_{\text{ND}}$ , Non-displaceable binding potential.

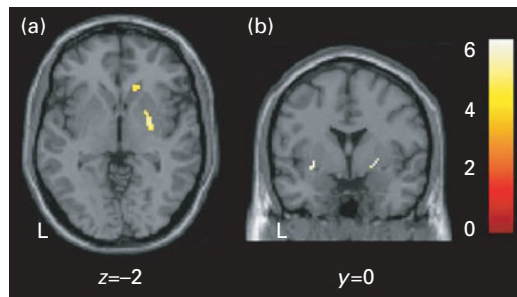
A three-way repeated-measure ANOVA revealed a significant drug  $\times$  group interaction.

<sup>a</sup> Post-hoc analysis revealed that overall  $\text{BP}_{\text{ND}}$  values of the striatal region in the nicotine condition were significantly lower than in placebo in smokers. The  $\text{BP}_{\text{ND}}$  value of the striatal region is the mean of pooled data across ROIs. There was no main effect of subject group ( $F_{1,10} = 0.12$ ,  $p = 0.74$ ).

performed using the simplified reference tissue model (Lammertsma and Hume, 1996). The cerebellum was used as reference region because it has been shown to be almost devoid of dopamine  $\text{D}_2$  receptors (Olsson et al., 1999; Suhara et al., 1999). The non-displaceable binding potential ( $\text{BP}_{\text{ND}}$ ) (Innis et al., 2007) values were analysed using a three-way repeated-measures ANOVA with subject group (smokers, non-smokers) as a between-subjects factor and drug (nicotine, placebo) and ROI as within-subjects factors. Statistical significance of  $p < 0.05$  was set for the analysis. To examine the relation between regional [ $^{11}\text{C}$ ]raclopride  $\text{BP}_{\text{ND}}$  and the degree of nicotine dependence, Pearson correlation coefficients between the  $\text{BP}_{\text{ND}}$  of each VOI of both nicotine and placebo conditions and the FTND score were calculated. In addition, in order to explore the relation between nicotine-induced dopamine release and nicotine dependence, correlations between the change in [ $^{11}\text{C}$ ]raclopride  $\text{BP}_{\text{ND}}$  of each VOI and FTND score were calculated. The threshold for significance was set at  $p = 0.05/8 = 0.006$  to avoid type 1 errors. To investigate detailed regions, parametric images of  $\text{BP}_{\text{ND}}$  were analysed using SPM (Gunn et al., 1997). Paired  $t$  tests were used to compare the  $\text{BP}_{\text{ND}}$  maps following nicotine and placebo administration in both groups. Subtracting the normalized  $\text{BP}_{\text{ND}}$  image in the nicotine condition from that in the placebo condition, we created individual  $\text{BP}_{\text{ND}}$  change maps. Regression analyses were conducted to examine the relation between  $\text{BP}_{\text{ND}}$  change and nicotine dependence.

## Results

Nicotine was not detected from any of the participants' plasma samples prior to the PET scans. During the PET scans, the plasma concentrations of nicotine using nicotine gum were 6–16 ng/ml, similar to those achieved by smoking a cigarette. There was no significant difference in the area under the nicotine plasma concentration–time curve (AUC) during PET scans between smokers and non-smokers.  $\text{BP}_{\text{ND}}$  of VOIs in both placebo and nicotine conditions are shown in Table 1. There was a significant drug  $\times$  subject group interaction ( $F_{1,10} = 6.42$ ,  $p = 0.03$ ). Post-hoc analysis revealed that  $\text{BP}_{\text{ND}}$  values of the striatal region in the nicotine condition were significantly lower than in placebo in smokers ( $F_{1,47} = 82.7$ ,  $p < 0.001$ ) but not in non-smokers ( $F_{1,47} = 1.99$ ,  $p = 0.17$ ). Result of voxel  $\times$  voxel parametric image analysis indicated significant  $\text{BP}_{\text{ND}}$  differences in the ventral caudate and putamen in smokers (Figure 1a). No significant correlation was found between the  $\text{BP}_{\text{ND}}$  of any VOI and FTND score in either the nicotine or placebo condition. However, the FTND score was correlated with the  $\text{BP}_{\text{ND}}$  difference between the two scanning sessions in the right ventral putamen ( $r = 0.961$ ,  $p = 0.002$ ). Trend-level correlations were observed between the FTND score and the  $\text{BP}_{\text{ND}}$  difference in the right ventral caudate ( $r = 0.911$ ,  $p = 0.012$ ) and the left ventral putamen ( $r = 0.907$ ,  $p = 0.012$ ). These correlations were also confirmed by parametric image analysis (Figure 1b). The  $\text{BP}_{\text{ND}}$  difference in the left ventral putamen



**Figure 1.** [ $^{11}\text{C}$ ]raclopride non-displaceable binding potential ( $\text{BP}_{\text{ND}}$ ) differences between the two scanning sessions in the striatum in smokers, and the correlation with nicotine dependence. (a) Image showing the significant [ $^{11}\text{C}$ ]raclopride  $\text{BP}_{\text{ND}}$  differences in the ventral caudate and putamen in smokers (height threshold at  $p < 0.005$ , uncorrected, and extent threshold of 10 voxels). (b) Image showing the correlation between the  $\text{BP}_{\text{ND}}$  differences in the ventral putamen and the Fagerstrom test for nicotine dependence (FTND) score (height threshold at  $p < 0.005$ , uncorrected, and extent threshold of 10 voxels). The bar shows the range of the  $t$  value. Within the images, L indicates left. Numbers in the bottom row indicate the coordinates of the Montreal Neurological Institute brain.

was also correlated with the reduction in craving score ( $r = 0.940$ ,  $p = 0.005$ ). There was no significant correlation between the  $\text{BP}_{\text{ND}}$  difference and the nicotine plasma concentration represented as AUC.

## Discussion

This is the first double-blind, randomized, placebo-controlled study to investigate dopamine release following nicotine administration in both smokers and non-smokers. Smokers showed significant decreases in [ $^{11}\text{C}$ ]raclopride  $\text{BP}_{\text{ND}}$  in the striatum in response to nicotine, and such decrease is thought to reflect the dopamine release following nicotine administration (Brody et al., 2004, 2006). In line with previous studies, there was no significant difference in striatal [ $^{11}\text{C}$ ]raclopride  $\text{BP}_{\text{ND}}$  between smokers and non-smokers in either the nicotine or placebo condition (Scott et al., 2007; Yang et al., 2006). However, only smokers showed significant decreases in [ $^{11}\text{C}$ ]raclopride  $\text{BP}_{\text{ND}}$  in the striatum, while non-smokers showed no detectable changes. The dopamine release in the ventral striatum was correlated with the degree of nicotine dependence and the reduction of craving score in smokers. Enhanced dopamine release in smokers might be a result of the reinforced effect of cigarette smoking. Two human PET studies (Barrett et al., 2004; Montgomery et al., 2007) reported no

overall changes in [ $^{11}\text{C}$ ]raclopride binding following nicotine administration in smokers. However, the majority of smokers in the latter study (Montgomery et al., 2007) were of low dependence and the plasma nicotine concentration was lower, whereas the majority of our smokers were moderately or highly dependent. In addition, those studies included female smokers, and gender differences in nicotine effects have been reported (Perkins et al., 1999).

As with other addictive drugs, animal studies have demonstrated that repeated nicotine administration enhances psychomotor responses, rewarding the effects of nicotine and striatal dopamine release in response to nicotine (Benwell and Balfour, 1992). Sensitization of the striatal dopamine response to nicotine has been implicated in the development of nicotine dependence (Benwell and Balfour, 1992).

Nicotinic acetylcholine receptors are expressed on both dopamine neurons and GABA neurons, and axon terminals of glutamatergic input to the midbrain (Laviolette and van der Kooy, 2004) and dopamine neurons in the midbrain are regulated by the balance of excitatory and inhibitory input to the midbrain (Mansvelder and McGehee, 2002). Chronic nicotine exposure was reported to reduce the sensitivity of GABA receptors and result in disinhibition of midbrain dopamine neurons (Amantea and Bowery, 2004). Chronic nicotine administration was also reported to increase the level of ionotropic glutamate receptors in the midbrain and conceivably enhance the excitatory input to the midbrain (Wang et al., 2007). Enhanced striatal dopamine release in smokers might be a consequence of altered control of dopamine release after repeated nicotine exposure.

In conclusion, compared to non-smokers, smokers showed enhanced striatal dopamine release in response to nicotine. The dopamine release in the ventral striatum following nicotine administration was correlated with the degree of nicotine dependence. Although this study is preliminary because of the limited sample, our findings were consistent with the report by Scott et al. (2007) with a similar sample size, suggesting both the feasibility of the nicotine gum method and the importance of the degree of dependence when examining the nicotine effect.

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## Statement of Interest

None.

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