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Nicotine Normalizes Increased Prefrontal Cortical Dopamine D_1 Receptor Binding and Decreased Working Memory Performance Produced by Repeated Pretreatment with MK-801: A PET Study in Conscious Monkeys

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The effects of acute nicotine were determined on dopamine (DA) D_1 (D_1R) and D_2 (D_2R) receptor binding in the neocortex of conscious monkeys under control conditions as well as after chronic pretreatment with MK-801 (dizocilpine), a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist. Extrastriatal neocortical D_1R and D_2R binding was evaluated with [^{11}C]NNC112 and [^{11}C]FLB457 with high-specific radioactivity using positron emission tomography (PET). Acute administration of nicotine bitartrate, given as an intravenous (i.v.) bolus plus infusion for 30 min at doses of 32 μg/kg + 0.8 μg/kg/min or 100 μg/kg + 2.53 μg/kg/min as base, induced slight but significant dose-dependent increases of DA in the extracellular fluid of prefrontal cortex (PFC) as determined by microdialysis. However**,** acute nicotine did not affect either [^{11}C]NNC112 or [^{11}C]FLB457 binding to D_1R or D_2R, respectively, in any cortical region. Chronic MK-801 (0.03 mg/kg, intramuscularly (i.m.), twice daily for 13 days) increased [^{11}C]NNC112 binding to D_1R in PFC. No significant changes were detected in [^{11}C]FLB457 binding to PFC D_2R. Although chronic MK-801 lowered baseline DA and glutamate levels in PFC, acute nicotine normalized reduced DA to control levels. Acute nicotine dose-dependently normalized the increased binding of [^{11}C]NNC112 to D_1R produced by chronic MK-801 but [^{11}C]FLB457 binding to PFC D_2R did not change. Working memory performance, impaired after chronic MK-801, was partially improved by acute nicotine. These results demonstrate that acute nicotine normalizes MK-801-induced PFC abnormality of D_1R in PFC.
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INTRODUCTION

Nicotine, the principal reinforcing component in tobacco smoke, binds to multiple nicotinic cholinergic receptors. Nicotine metabolites and other substances in tobacco smoke have been suggested to enhance dopamine (DA) release (Crooks and Dwoskin, 1997). Tobacco smoking also suppresses monoamine oxidase (MAO A and B) activity (Fowler *et al*, 1996, 1998). The rewarding/reinforcing actions of nicotine/tobacco are attributed to stimulation of the dopaminergic mesolimbic system and brain reward pathways (Nisell *et al*, l995; Gardner, 1997; Pontieri *et al*, 1997). Like other abused substances, nicotine increases extracellular DA levels in rat nucleus accumbens (Imperato *et al*, 1986; Di Chiara and Imperato, 1988; Damsma *et al*, 1989; Nisell *et al*, 1994a, b, 1995). Tobacco smoke and nicotine also increase DA utilization in rat nucleus accumbens (Fuxe *et al*, 1986). Lesions of dopaminergic nerve terminals in rat nucleus accumbens decrease nicotine self-administration (Singer *et al*, 1982; Corrigall *et al*, 1992) and nicotine-induced rat locomotor stimulation (Clarke *et al*, 1988).

Involvement of the dopaminergic system in prefrontal cortex (PFC) has been suggested as another neurochemical pathway of nicotine action (Marshall *et al*, 1995). The incidence of tobacco smoking in schizophrenic patients varies from 50% to about 90%, which is greater than the rate of smoking in the general population (Matherson and O'Shea, 1984; Goff *et al*, 1992; Levin *et al*, 1997; Dalack *et al*, 1998; Brown *et al*, 2000). Schizophrenia is associated with a dysregulation of DA function in both the PFC and striatum

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(for reviews see Cho and Lewis, 2004; Berman and Meyer-Lindenberg, 2004). Glassman (1993) suggested that nicotine in tobacco, by releasing DA, may reduce a dopaminergic deficiency in psychiatric patients who smoke. Amphetamine produces a larger displacement of the D_2 radiolabeled receptor ligand raclopride in the striatum of schizophrenic patients than mentally normal controls (Laruelle *et al*, 1996; Brier *et al*, 1997; Abi-Dargham *et al*, 1998). Both hypo- and hyperfunction of the PFC has been reported. Schizophrenic patients with negative symptoms have impaired working memory performance. Hypodopaminergic activity in the PFC has been proposed as the cause (Davis *et al*, 1991). Some of the beneficial effects of smoking and nicotine may be due to an action in the PFC (Dursun and Kutcher, 1999).

Noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists including MK-801, ketamine, and phencyclidine in low concentrations bind to the ion channel associated with the NMDA receptor (Thomson *et al*, 1985; Fagg, 1987; Johnson and Jones, 1990; Moriyoshi *et al*, 1991; Dingledine *et al*, 1999). These agents markedly impair cognition including working memory performance in healthy humans and resemble symptoms of schizophrenia (Luby *et al*, 1959; Snyder, 1980; Weinberger *et al*, 1986; Javitt and Zukin, 1991; Krystal *et al*, 1994; Malhotra *et al*, 1996; Jentsch and Roth, 1999; Domino *et al*, 2004). When a noncompetitive NMDA receptor antagonist is administered to experimental animals, hyperlocomotion and stereotyped behavior are induced (Johnson and Jones, 1990). These effects on animals have been used as a model for psychosis in humans. We have previously reported that chronic administration of MK-801 in monkeys impairs working memory performance, which is closely related to functions of the PFC (Tsukada *et al*, 2005).

Positron emission tomography (PET) noninvasively measures the neuroanatomical distribution of radiolabeled dopamine-specific ligands in living brain. Recently, [^{11}C]NNC112 (Halldin *et al*, 1998) and [^{11}C]FLB457 (Halldin *et al*, 1995) have been developed to assess extrastriatal DA D_1 (D_1R) and D_2 receptors (D_2R), respectively. Both radioligands have enough high affinity to assess the lower density of DA receptors in extrastriatal regions (Hall *et al*, 1988; Lidow *et al*, 1989). Chronic phencyclidine and ketamine abusers have increased D_1 receptor binding in PFC as measured with [^{11}C]NNC112 (Abi-Dargham *et al*, 2000, 2003; Abi-Dargham and Moore, 2003). Chronic administration of MK-801 results in abnormally increased D_1R binding with [^{11}C]NNC112 in the PFC accompanied by impaired working memory performance in conscious monkeys (Tsukada *et al*, 2005).

The aim of the present study was to evaluate the effects of acute nicotine on MK-801-induced impairment of DA neuronal system in PFC using PET as well as on impaired working memory performance in conscious monkeys. In addition, the effects of MK-801 and/or acute nicotine on DA and glutamate release in PFC were assessed using microdialysis.

MATERIALS AND METHODS

Animals and Drugs

A total of 15 adult male rhesus monkeys (*Macaca mulatta*), weighing 5.4–7.2 kg, were randomly assigned to each saline ($n=5$), 'Low' ($n=5$), and 'High' ($n=5$) nicotine groups. The monkeys were maintained and handled in accordance with the recommendations of the US National Institutes of Health and the Guidelines of the Central Research Laboratory of Hamamatsu Photonics KK. Magnetic resonance images (MRI) of each pentobarbital anesthetized monkey were obtained with a Toshiba MRT-50A/II (0.5 T). The animals were trained to sit in a monkey chair several days per week for more than 3 months. At least 1 month before the PET study, an acrylic plate was attached to the skull under pentobarbital anesthesia (Onoe *et al*, 1994). Subsequently, after each monkey recovered, the plate was fixed to a monkey chair for the PET study. The stereotactic coordinates of PET and MRI were adjusted based on the orbitomeatal (OM) line with a specially designed head holder.

Nicotine bitartrate was purchased from Kanto Chemical (Tokyo, Japan). (+)-MK-801 was purchased from RBI (Natick, MA). FLB457 and the precursor of [^{11}C]FLB457 were obtained from ABX (Dresden, Germany). NNC112 and the precursor of [^{11}C]NNC112 were gifts from Professor Christer Halldin of the Karolinska Institute, Sweden. Nicotine and MK-801 were diluted in 0.9% saline.

Drug Treatments

In order to induce dysfunction of the PFC dopaminergic neuronal system, as previously reported (Tsukada *et al*, 2005), MK-801 at a dose of 0.03 mg/kg was administered intramuscularly (i.m.) twice a day for 13 days. For the acute study, 30 min before injection of [^{11}C]NNC112 or [^{11}C]FLB457, saline or MK-801 (0.03 mg/kg) was given intravenously (i.v.) On the 7th and 14th days after the beginning of chronic administration, microdialysis analyses of DA and glutamate and PET scans with [^{11}C]NNC112 and [^{11}C]FLB457 were performed with an interval of at least 15 h after the last MK-801 administration.

Nicotine bitartrate was then given as an i.v. bolus plus infusion for 30 min in doses of 32 μg/kg + 0.8 μg/kg/min or 100 μg/kg + 2.53 μg/kg/min as base, then the first PET measurement was begun.

Syntheses of [^{11}C]NNC112 and [^{11}C]FLB457

Carbon-11 (^{11}C) was produced by a ^{14}N(p,α)^{11}C nuclear reaction using a cyclotron (HM-18, Sumitomo Heavy Industry, Tokyo, Japan) at Hamamatsu Photonics PET Center and obtained as [^{11}C]CO_2. This was converted to [^{11}C]methyl iodide via [^{11}C]methane using PET trace MeI MicroLab (GE Medical Systems, Milwaukee, WI).

[^{11}C]NNC112 (Halldin *et al*, 1998) was labeled with ^{11}C by *N*-methylation of its nor-compound with [^{11}C]methyl iodide. [^{11}C]FLB457 (Halldin *et al*, 1995) was labeled with ^{11}C by *O*-methylation of its nor-compound. The radiochemical and chemical purities of labeled compounds used were greater than 98 and 99%, respectively. The specific radioactivity ranged from 315 to 420 GBq/μmol for [^{11}C]NNC112, and from 360 to 402 GBq/μmol for [^{11}C]FLB457. After HPLC analysis for identification and purity, the solution was passed through a 0.22 μm pore filter before i.v. administration to each monkey.

PET Measurement and Analysis

Data were collected on a high-resolution PET scanner (SHR-7700, Hamamatsu Photonics, Hamamatsu, Japan) with a transaxial resolution of 2.6 mm full-width at half-maximum (FWHM) and a center-to-center distance of 3.6 mm (Watanabe *et al*, 1997). The PET camera allowed 31 slices for imaging to be recorded simultaneously.

After an overnight fast, each animal was placed in the monkey chair with stereotactic coordinates aligned parallel to the OM line. PET scans with [^{11}C]NNC112 and [^{11}C]FLB457 were performed in the 3D data acquisition mode for 64 min with six time frames at 10 s intervals, six time frames at 30 s, 12 time frames at 1 min, followed by 16 time frames at 3 min. Injected radioactivity was ca 10 MBq/kg body weight for each ligands. PET scans with [^{11}C]NNC112 and [^{11}C]FLB457 were performed in a counterbalanced order with a 2-h interval between scans.

For quantitative analysis, time–activity curves of radioactivity in the cerebellum were used as an input function because of its much lower density of DA receptors (Creese *et al*, 1975). Each region of interest (ROI) was fitted to a two-compartment model using the least-square fitting method to estimate the kinetic parameters (K_1 and k_2). The distribution volume (DV) in each ROI was calculated as the ratio of K_1/k_2 (Lammertsma and Hume, 1996). The ratio of the brain tissue to the blood concentration is called the DV or the partition coefficient. It can be thought of as the volume of 1 ml of blood that at equilibrium contains the same amount of radioactivity as 1 g of tissue.

Microdialysis Analysis

A guide cannula was previously implanted 35 mm anterior to the intrameatal line and 10 mm lateral from the midline (A: 35, L: 10) according to the individual MR images. A microdialysis probe with a membrane region of 250 μm in diameter and 3 mm in length (Eicom A-I-8-03, Eicom, Tokyo, Kyoto, Japan) was inserted (only when scheduled) into the PFC (3.0 mm below the dura matter) of the monkey brain via the guide cannula. The probe was initially perfused with a modified Ringer solution (147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$, Otsuka Pharmaceutical, Tokyo, Japan) at a rate of 10 μl/min to remove overflow of neurotransmitters from the damaged tissue. The perfusion rate was decreased to 5 μl/min 2 h after insertion of the probe. In all, 75 μl samples were collected every 15 min, and extracellular fluid (ECF) DA and glutamate contents were measured by HPLC systems (HTEC-500 and DTA-300, Eicom, Kyoto, Japan; Kino *et al*, 2004). The microdialysis probes remained implanted throughout the duration of the chronic treatments.

Microdialysis analyses were performed before chronic administration of MK-801 ('Control' condition), and after chronic MK-801 treatment for 13 days ('MK-801' condition). The mean data obtained from 0 to 120 min before administration of nicotine were used as 'baseline' data. Nicotine (32 μg/kg + 0.8 μg/kg/min for 30 min or 100 μg/kg + 2.53 μg/kg/min for 30 min as base) was administered 120 min after the start of the sampling. The levels of DA and glutamate in ECF of PFC were expressed as '% of baseline'.

Behavioral Tasks

Behavioral task performance was evaluated as described previously (Inoue *et al*, 2004; Tsukada *et al*, 2004, 2005). Briefly, in the oculomotor delayed response (ODR) task, after a short inter-trial interval (ITI), a small red spot (0.1° in diameter) appeared as a fixation point at the center of a 15-in monitor placed in front of the monkey 57 cm from its face. Each highly trained monkey was required to look at the fixation point and maintain fixation. The monkey's horizontal and vertical eye positions were recorded at 60 Hz by a monitoring system using an infrared camera (X-Y Tracer C3162, Hamamatsu Photonics, Hamamatsu, Japan). After the monkey maintained fixation for 1 s, a red circle (0.5° in diameter) was presented as a target cue for 100 ms (cue period), which was randomly presented at one of eight predetermined positions. Eccentricity was 5° from the fixation point. The monkey was required to maintain fixation at the fixation point during the cue period and the subsequent 0.5–10 s delay period. At the end of the delay period, the fixation point was extinguished. The monkey was trained to make a saccade to the position where the target cue had been presented. If the monkey made a correct saccade within 500 ms, it was rewarded with a drop of water.

In the visually guided saccade (VGS) task, after a short ITI, a fixation point appeared at the center of the monitor. The monkey was required to look at the fixation point and maintain it. After the monkey maintained fixation for 1 s, the fixation point was extinguished and a target cue was presented at one of eight predetermined positions. When the target cue was presented, the monkey had to make a saccade to the target cue within 500 ms. ODR and VGS task data were obtained in 20 trials for each condition. The means ± SD were used for further data analysis.

Behavioral analyses were performed before chronic administration of MK-801 ('Control' condition), and after chronic MK-801 treatment for 13 days ('MK-801' condition). Vehicle or nicotine (32 μg/kg + 0.8 μg/kg/min for 30 min or 100 μg/kg + 2.53 μg/kg/min for 30 min as base) was administered 35 min prior to the behavioral analysis. In the assessments of drug treatments, the delay period between cue presentation and saccade timing was fixed at 6 s.

Statistical Analysis

Results are expressed as means ± SD. Comparisons were carried out using unpaired, two-tailed Student's *t*-test. A probability level of less than 5% ($P<0.05$) was considered significant.

RESULTS

Figure 1 illustrates the experimental protocol showing the time sequence of microdialysis, behavioral task, and PET scans in monkeys. In general, these three studies were performed in parallel.

Typical MRI and PET images of [^{11}C]NNC112 for D_1R and [^{11}C]FLB457 for D_2R in the conscious monkey brain are shown with ROIs in Figure 1 of Tsukada *et al* (2005). Accumulation of [^{11}C]NNC112 and [^{11}C]FLB457 was high in the striatum, medium in the cortical regions, and low in the

cerebellum when accumulated images were created 45 min and later after tracer injection. The maximum accumulation of [^{11}C]NNC112 was observed 10 min postinjection in the occipital cortex (OCC), 15 min after injection in the PFC and the temporal cortex (TMC), and decreased gradually thereafter. The time–activity curve of [^{11}C]FLB457 indicated that the peaks of radioactivity in the cortical regions were 5 min postinjection in OCC, 10 min postinjection in PFC and TMC, and decreased thereafter. In the cerebellum, the time–activity curves of [^{11}C]NNC112 and [^{11}C]FLB457 peaked within 5 min postinjection, followed by a gradual decrease with time (data not shown).

In order to evaluate the effects of acute administration of nicotine on [^{11}C]NNC112 and [^{11}C]FLB457 binding *in vivo*, monkeys were given i.v. nicotine in a bolus dose of 32 μg/kg and an infusion dose of 0.8 μg/kg/min for 30 min ('Low' dose condition), or 100 μg/kg bolus and 2.53 μg/kg/min for 30 min ('High' dose condition). We have previously reported that both doses of nicotine produced arterial blood levels in monkeys in the range of tobacco smoking in humans (Tsukada *et al*, 2002). Neither dose of nicotine had any effects on the *in vivo* distribution volume ($DV = K_1/k_2$) of [^{11}C]NNC112 to D_1R (Figure 2a) or [^{11}C]FLB457 to D_2R (Figure 2b) in any regions of the monkey brain.

Acute i.m. administration of MK-801 in a dose of 0.03 mg/kg did not affect the *in vivo* binding of [^{11}C]NNC112 (Figure 3a) or [^{11}C]FLB457 (Figure 3b) in any cortical region. During chronic treatment with MK-801 (0.03 mg/kg i.m., twice a day for 13 days), [^{11}C]NNC112 binding to D_1R significantly increased on the 14th day in PFC, but not in TMC and OCC (Figure 3a). In contrast, [^{11}C]FLB457 binding to D_2R showed no significant changes in any cortical regions on the 7th day, and the slight tendency to increase, but did not reach significance in PFC on the 14th day (Figure 3b).

When nicotine was acutely administered at 'Low' and 'High' doses in monkeys treated chronically with MK-801, the increased PFC [^{11}C]NNC112 binding to D_1R was dose-dependently decreased to the normal level observed before the beginning of chronic MK-801 treatment (Figure 4). Nicotine did not affect [^{11}C]NNC112 binding in TMC and OCC (data not shown). In contrast, neither dose of nicotine induced any changes in [^{11}C]FLB457 binding to D_2R in any region (Figure 4).

The effects of acute nicotine administration on DA and glutamate levels in the PFC ECF were evaluated by microdialysis in conscious monkey brain before (control) and after chronic MK-801 treatment as shown in Figures 5 and 6. The baseline DA level was 0.52 ± 0.07 fmol/μl in PFC. The DA levels in the PFC ECF slightly but significantly increased in a dose-dependent manner when acute nicotine was i.v. administered at 'Low' and 'High' doses to normal monkeys (147.2 and 208.4%, respectively, of baseline; Figure 5a). DA levels peaked just after nicotine injection,

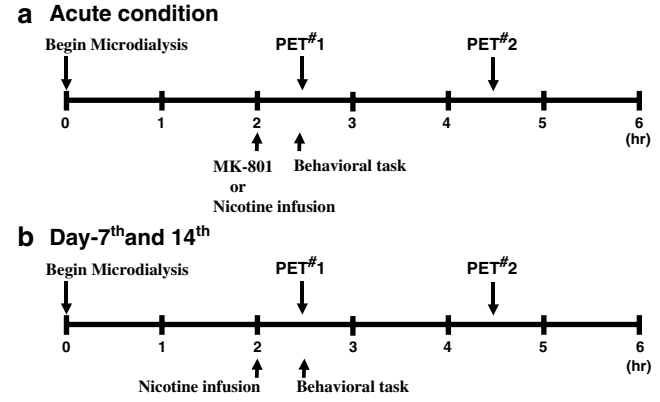

Figure 1 Protocol for microdialysis, behavioral task and PET scans in monkeys in acute (a) and chronic MK-801 treatment (b) conditions. (a) Microdialysis analysis was begun at time-0, followed by i.v. administration of MK-801 or infusion of nicotine. At 30-min postadministration, the behavioral test and the first PET scan were begun simultaneously. The second PET scan was performed 2 h after the first one. (b) Microdialysis collection continued and nicotine infusion were performed as shown. MK-801 was administered at least 15 h before time-0.

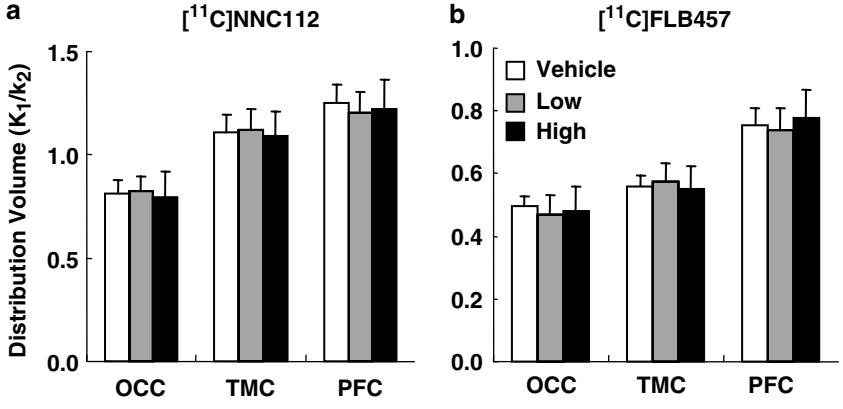

Figure 2 Lack of effects of acute nicotine on [^{11}C]NNC112 (a) and [^{11}C]FLB457 (b) binding in the control conscious monkey brain. Nicotine was given in an i.v. bolus dose of 32 μg/kg and an infusion dose of 0.8 μg/kg/min ('Low' dose) and 100 μg/kg bolus and 2.53 μg/kg/min ('High' dose) for 30 min, then PET scans were performed. The regions of interest (ROIs) are the same as those published in Figure 1 of Tsukada *et al*. (2005) for this and subsequent figures. Time–activity curves of radioactivity in the cerebellum and each ROI were fitted to a two-compartment model using the least-squares method. The distribution volume ($DV = K_1/k_2$) in each ROI was calculated. OCC, occipital cortex; TMC, temporal cortex; PFC, prefrontal cortex; the bar graphs represent mean ± SD in this and subsequent figures.

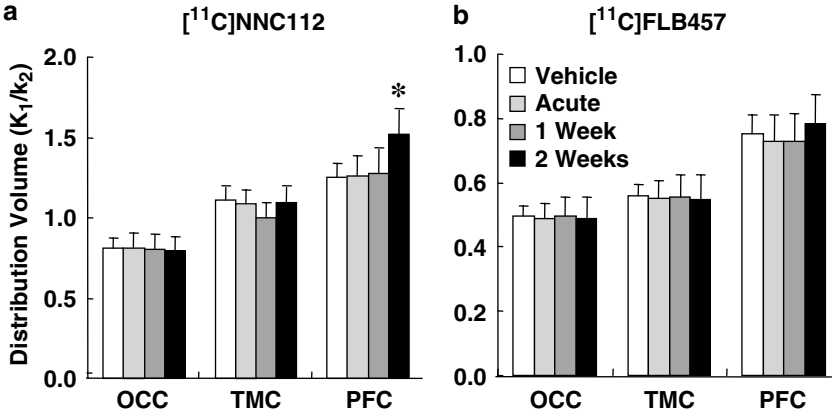

Figure 3 Effects of MK-801 on the binding of [^{11}C]NNC112 (a) and [^{11}C]FLB457 (b) in the conscious monkey brain. In the acute condition, 30 min before injection of [^{11}C]NNC112 or [^{11}C]FLB457, vehicle or MK-801 (0.03 mg/kg, i.m.) was administered i.v. In the chronic condition, MK-801 (0.03 mg/kg, i.m.) was administered twice a day for 13 days. On the 14th day, PET scans were performed with an interval of at least 15 h after the last dose of MK-801. The distribution volume ($DV = K_1/k_2$) in each ROI was calculated as described above. *$P < 0.05$ *vs* 'Vehicle' condition.

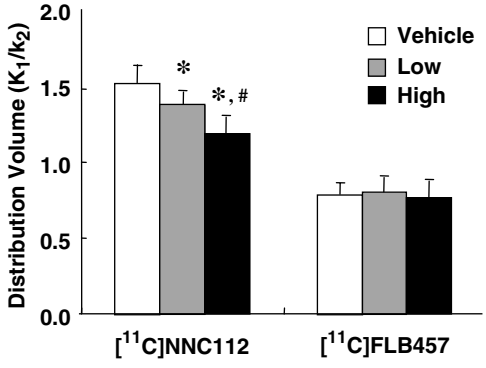

Figure 4 Effects of acute nicotine on [^{11}C]NNC112 (a) and [^{11}C]FLB457 (b) binding in the chronically MK-801-treated conscious monkey brain. Nicotine was given in an i.v. bolus dose of 32 μg/kg and an infusion dose of 0.8 μg/kg/min ('Low' dose) and 100 μg/kg bolus and 2.53 μg/kg/min ('High' dose) for 30 min, then PET scans were performed as shown in Figure 1. The distribution volume ($DV = K_1/k_2$) in the PFC was calculated as described above. *$P < 0.05$ *vs* 'Vehicle' condition, #$P < 0.05$ *vs* 'Low' dose condition.

followed by a gradual return to the baseline level (Figure 5a). After chronic treatment with MK-801 (0.03 mg/kg i.m., twice a day for 13 days), the baseline levels of DA in PFC were reduced to ca 60% (0.31 ± 0.04 fmol/μl) of control levels (Figure 5b). Acute nicotine dose-dependently increased the PFC ECF DA levels, almost reaching the control baseline at 'Low' dose (0.51 ± 0.03 fmol/μl) and greater at 'High' dose (0.66 ± 0.09 fmol/μl; Figure 5b). The magnitude of DA release after chronic MK-801 treatment was greater (147.2 *vs* 165.2% at 'Low' dose; 208.4 *vs* 229.1% at 'High' dose at peak time point) and more prolonged (AUC; 127.4 and 132.2%, respectively, of control at 'Low' and 'High' doses) with nicotine administration than that observed in the control state (Figure 5a and b).

The baseline glutamate level (1.32 ± 0.25 fmol/μl) in PFC was significantly increased in a dose-dependent manner when acute nicotine was administered to normal monkeys (115.1 and 131.9%, respectively, of baseline) (Figure 6a). The peak time points were observed between 45 and 60 min postnicotine administration, showing slight delay compared to DA peak time. After chronic treatment with MK-801, the baseline glutamate levels were reduced to ca 40% (0.50 ± 0.06 fmol/μl) of control levels (Figure 6b). Acute nicotine dose-dependently increased the glutamate levels to 139.5 and 175.7%, respectively, at 'Low' and 'High' doses (Figure 6b), which were greater than that induced in the control state shown in Figure 6a. In contrast, the release of both DA and glutamate did not change with chronic saline treatment.

Before chronic MK-801 treatment, a delay-dependent reduction in the correct response was observed in ODR task performance, showing 79% accuracy at a 6-s delay period with vehicle treatment, while no significant on VGS task performance was determined (Figure 7a). Acute nicotine administration at 'Low' and 'High' doses produced no significant changes in the ODR or VGS tasks with a similar 6-s delay period (Figure 7a). On the 14th day, postchronic MK-801 administration, ODR task performance, with a 6-s delay period, showed marked impairment (Figure 7b). Acute administration of nicotine at 'Low' dose showed a tendency for improvement of ODR task performance, but did not reach statistical significance. Nicotine administration at 'High' dose significantly reversed the impaired ODR task performance induced by chronic MK-801 treatment (Figure 7b). VGS task performance was not influenced by vehicle or acute nicotine post-MK-801 treatment (Figure 7b).

DISCUSSION

A key methodological issue raised by one of the reviewers of this paper is the relative sensitivity to displacement by DA for the two new labeled ligands used herein, NNC (D_1R) and FLB (D_2R) compared to raclopride (D_2R). When [^{11}C]raclopride and [^{11}C]FLB457 binding to DA D_2 receptors is compared, [^{11}C]raclopride is more sensitive to alterations of synaptic DA levels because of its much lower affinity

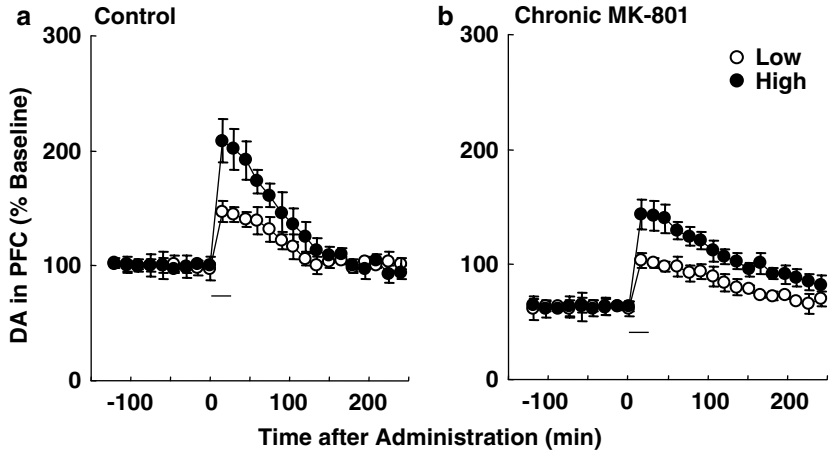

Figure 5 Effects of acute nicotine on dopamine release in the extracellular fluid (ECF) of PFC in the conscious monkey before (a) and after chronic MK-801 treatment (b). A microdialysis probe was inserted into PFC region via a guide cannula. Samples were collected every 15 min at a rate of 5 μl/min. The DA concentration was analyzed by HPLC. At time-0, nicotine was given as an i.v. bolus of 32 μg/kg and an infusion dose of 0.8 μg/kg/min ('Low' dose) and 100 μg/kg bolus and 2.53 μg/kg/min ('High' dose) for 30 min as shown by the horizontal black bars. Mean values obtained from 0 to 120 min in each condition were used as 'baseline'. Dopamine concentrations were expressed as '% baseline' of the 'Control.'

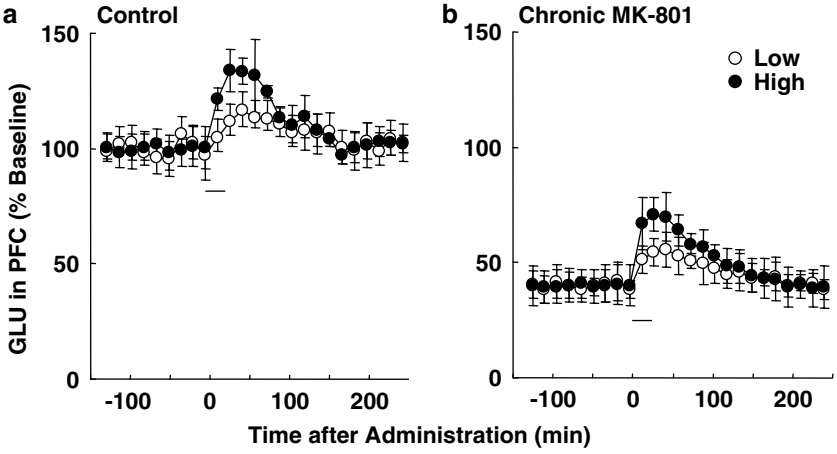

Figure 6 Effects of acute nicotine on glutamate release in the extracellular fluid (ECF) of PFC in the conscious monkey before (a) and after chronic MK-801 treatment (b) Microdialysis analysis was performed as described in the legend of Figure 5. At time-0, nicotine was given in an i.v. bolus of 32 μg/kg and an infusion dose of 0.8 μg/kg/min ('Low' dose) and 100 μg/kg bolus and 2.53 μg/kg/min ('High' dose) for 30 min as shown by the horizontal black bars. Mean values obtained from 0 to 120 min in each condition were used as 'baseline'. Glutamate concentrations were expressed as '% baseline' of the 'Control' condition.

(Ki = 1.2 nM) to the receptors than [^{11}C]FLB457 (18 pM), which is not displaced by increased DA-induced release by methamphetamine (Okauchi *et al*, 2001). In DA D_1 receptor binding, [^{11}C]NNC112 and [^{11}C]SCH23390 have similar affinity (0.2 and 0.4 nM, respectively); [^{11}C]SCH23390 binding is not affected by methamphetamine (Tsukada *et al*, 2001a). Taken together, the order of sensitivity to displacement by DA is: [^{11}C]raclopride ⋙ [^{11}C]FLB457 = [^{11}C]NNC112. It should be noted that intrasynaptic DA concentrations are not the only factor to modulate ligand-receptor binding *in vivo* (for details see Tsukada *et al*, 1999a, 2000a, b).

The present results demonstrate that repeated daily MK-801 treatment induced hypoactivation of dopaminergic neuronal transmission, upregulation of D_1R, but not D_2R, binding in the PFC and impairment of working memory performance in monkeys but did not change their gross behavior. These alterations were normalized by acute administration of nicotine in doses producing similar blood levels as tobacco smoking in humans.

As described in the Introduction, NMDA receptor antagonists have been reported to impair cognitive functions and resemble the symptoms of schizophrenia (Luby *et al*, 1959; Snyder, 1980; Weinberger *et al*, 1986; Javitt and Zukin, 1991; Krystal *et al*, 1994; Malhotra *et al*, 1996; Jentsch and Roth, 1999; Domino *et al*, 2004). PET studies have suggested that dysfunction of the extrastriatal dopaminergic system exists in schizophrenic patients (Okubo *et al*, 1997; Lindström *et al*, 1999; Abi-Dargham *et al*, 2002; Suhara *et al*, 2002; Laruelle *et al*, 2003). We have found that acute and chronic NMDA antagonism impairs cognitive function through different modulations in the dopaminergic neuronal system in the PFC of monkeys (Tsukada *et al*, 2005). Thus, acute systemic administration of low doses (0.03 and

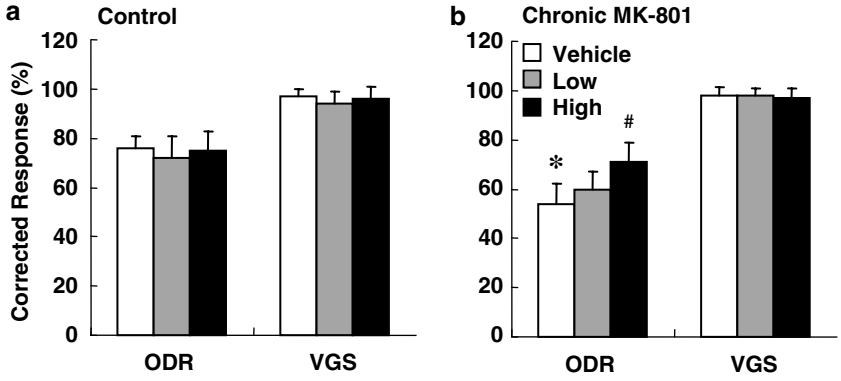

Figure 7 Effects of acute nicotine on working memory performance on conscious monkeys before ('Control', a) and after chronic MK-801 treatment (b). Working memory performance was evaluated with the oculomotor delayed response (ODR) task and visually guided saccade (VGS) task with an acute dose of vehicle or nicotine i.v. of 32 μg/kg and an infusion dose of 0.8 μg/kg/min ('Low' dose) and 100 μg/kg bolus and 2.53 μg/kg/min ('High' dose) for 30 min before and after MK-801 administration in a dose of 0.03 mg/kg, i.m. twice a day for 13 days. *$P<0.05$ *vs* 'Vehicle' in ODR of Control condition, #$P<0.05$ *vs* 'Vehicle' in ODR of chronic MK-801.

0.1 mg/kg, i.m.) of MK-801 increased glutamate and DA levels, while chronic MK-801 treatment lowered basal glutamate and DA levels in the ECF of PFC. Acute MK-801 reduced [^{11}C]FLB457 binding to D_2R (in larger doses than used herein), but not [^{11}C]NNC112 binding to D_2R in PFC (Tsukada *et al*, 2005). In contrast, chronic MK-801 induced increased D_1R binding without any changes in D_2R binding in PFC. Interestingly, both acute and chronic MK-801 treatments impaired cognitive function of monkeys as assayed by an ODR task in the present and previous studies (Tsukada *et al*, 2005). These data indicate that proper functioning of the DA system in PFC is important for working memory-related tasks. With repeated MK-801 treatment in a dose of 0.03 mg/kg twice a day for 13 days, the degree of impaired ODR task performance provided a significant inverse correlation with upregulated [^{11}C]NNC112 binding to D_1R (Tsukada *et al*, 2005). These results confirm the significant roles of PFC D_1R activity in working memory as previously suggested (Sawaguchi and Goldman-Rakic, 1991). The now classic studies by Goldman-Rakic and colleagues regarding the role of the PFC and working memory in non-human primates and relevance to schizophrenia (Goldman, 1971; Goldman-Rakic, 1994; Smiley and Goldman-Rakic, 1993) is a solid foundation on which the present research merely adds additional support. Regarding D_1R binding in PFC of schizophrenic patients, two apparently discordant results have been published using PET. One demonstrated decreased D_1R binding assayed with [^{11}C]SCH23390 (Okubo *et al*, 1997), while the other observed increased binding measured with [^{11}C]NNC112 (Abi-Dargham *et al*, 2002). In view of the fact that [^{11}C]SCH23390 binding is reduced with DA depletion, both studies suggest that PFC D_1R binding is produced by hypodopaminergic neuronal transmission. The present microdialysis data actually demonstrate decreased DA release in PFC after chronic MK-801 treatment. Decreased DA neuronal activity, therefore, contributes to the increased D_1R binding in PFC. There is no direct evidence that MK-801 changes the affinity of D_1 receptors but changes in DA levels probably do. These results suggest that the neuronal mechanisms by which noncompetitive NMDA receptor antagonists activate DA transmission contribute to long-term reduction of dopaminergic activity, resulting in cognitive deficits.

It is of interest that acute nicotine administration at the 'High' dose improved working memory performance, which was impaired after chronic MK-801 treatment in the monkeys. In addition, the acute nicotine treatment dose-dependently normalized chronic MK-801-induced increases in [^{11}C]NNC112 binding to D_1R in PFC. When nicotine was administered in either dose to the monkeys before chronic MK-801 treatment, no significant changes were observed in the working memory performance or in the binding [^{11}C]NNC112 to D_1R in PFC. This suggests that nicotine might act to normalize lowered PFC dopaminergic neuronal activity. Using microdialysis analysis, these doses of acute nicotine produced a slight but significant elevation of DA levels in the ECF in a dose-dependent manner. It has been assumed that elevated DA levels in the synaptic cleft result in reduced binding of a competitive radiolabeled ligand to its specific binding site. This phenomenon was observed in the interactions between [^{11}C]raclopride and D_2R in the striatum (for a review, see Laruelle, 2000). Our previous results, however, demonstrated that unlike methamphetamine, nicotine in similar doses as used herein did not affect [^{11}C]raclopride binding to D_2R in the striatum. This indicates that there is too small an elevation of striatal DA to produce change in [^{11}C]raclopride binding *in vivo* (Tsukada *et al*, 2002). Owing to the much greater affinity of [^{11}C]NNC112 to D_1R or [^{11}C]FLB457 to D_2R than that of [^{11}C]raclopride to D_2R, such binding appears to be insensitive to a small increase in DA induced by nicotine in the control conscious state. However, after chronic MK-801 administration for 13 days, accompanied by reduced baseline levels of glutamate, baseline DA levels were lowered to ca 60% of the control baseline before MK-801 treatment in the same animals. This is a very important observation that indicates a hypodopaminergic neuronal activity was produced after chronic MK-801. Our previous results with microdialysis also showed that chronic MK-801 treatment first decreased glutamate release in the PFC followed by reduced DA release (Tsukada *et al*, 2005). These findings

are consistent with the present data. Of interest, in chronic MK-801-treated monkeys, acute nicotine normalized DA release to almost the control level or more with higher magnitudes of release levels and with a longer duration period compared to normal monkeys. On the contrary, although dose-dependent glutamate release was also induced by acute nicotine, the magnitude and duration period were almost similar between control and chronic MK-801-treated monkeys. These results suggest that acute nicotine affects dopaminergic and glutamatergic neuronal systems in an independent manner. Drew *et al* (2000) hypothesized that DA transporter (DAT)-mediated DA release by nicotine was via $\alpha 4\beta 2$ receptors in rat PFC. They found that nicotine further enhanced amphetamine-stimulated DA release in PFC, suggesting that an activated DAT facilitated DA release by nicotine. Whether or not the basal activity of the DAT is activated after chronic MK-801 treatment is not known. There is no evidence that nicotine can act directly on the DAT. However, recent evidence suggests that modulation of several receptors on dopaminergic terminals alters DAT activity (Meiergard *et al*, 1993; Ichikawa *et al*, 1995; Yamashita *et al*, 1995; Izenwasser *et al*, 1998; Drew *et al*, 2000; Tsukada *et al*, 1999a, b, 2000a, b, 2001a, b). In addition, it has been shown that NMDA antagonism increased DAT availability in the monkey striatum as measured by [^{11}C]β-CFT (Tsukada *et al*, 2000a, 2001a). Our results indicate that acute nicotine administration facilitates dopaminergic neuronal transmission previously suppressed by chronic MK-801 treatment. Although several possible molecular mechanisms can be proposed, nicotine reduces the increased PFC [^{11}C]NNC112 binding to D_1R without interactive modulations with the glutamatergic NMDA neuronal system.

In conclusion, the present results indicate that acute nicotine, in tobacco smoking-related doses, normalizes the impaired working memory performance related to hypodopaminergic neuronal function as evidenced by lower ECF DA release and increased D_1R binding in the PFC of chronic MK-801-treated monkeys. This finding may explain, in part, the persistence of tobacco smoking among schizophrenic patients who have cognitive deficits due to low dopaminergic activity in PFC.

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