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Chronic NMDA Antagonism Impairs Working Memory, Decreases Extracellular Dopamine, and Increases D_1 Receptor Binding in Prefrontal Cortex of Conscious Monkeys

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This study demonstrates that dizocilpine (MK-801), a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, impairs working memory of conscious behaving monkeys. In addition, acute and chronic MK-801 produces different effects on D_1 and D_2 receptor binding in prefrontal cortex (PFC). Extrastriatal neocortical receptor D_1 (D_1R) and D_2 (D_2R) binding were assayed by [^{11}C]NNC112 and [^{11}C]FLB457, respectively, using high-specific radioactivity and a specially designed monkey positron emission tomograph (PET). Acute single dose (0.03, 0.1, and 0.3 mg/kg) i.v. administration of MK-801 resulted in dose-related impairment of working memory performance of an oculomotor delayed response (ODR) task. There was no impairment of performance of a visually guided saccade (VGS) task with low doses of 0.03 and 0.1, but it was depressed with 0.3 mg/kg. Chronic daily MK-801 (0.03 mg/kg, i.m., b.i.d. for 13 days) induced impaired ODR task performance with no effect on the VGS task. Although acute single doses of MK-801 caused no significant changes in [^{11}C]NNC112 binding to PFC D_1R, chronic daily treatment increased binding about 14% ($P<.05$). Acute MK-801 dose-dependently decreased [^{11}C]FLB457 binding about 35% ($P<.01$) to PFC D_2R; chronic treatment had no significant effect. Microdialysis analyses demonstrated that acute single doses of MK-801 (0.03 and 0.1 mg/kg) increased extracellular glutamate and dopamine (DA) levels in PFC. Chronic MK-801 gradually lowered glutamate and DA levels in PFC. The results demonstrate in conscious, unanesthetized primates that MK-801 induces impairment of PFC function, as measured by working memory performance. Furthermore, in response to lowered levels of DA in PFC, D_1R binding is increased, whereas D_2R binding is not.
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INTRODUCTION

Working memory, which is the brief retention of internalized information to guide behavior, is known to be related to the prefrontal cortex (PFC) function (Goldman-Rakic, 1987). Noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists, MK-801, ketamine, and phencyclidine, bind to an ion channel associated with the NMDA receptor (Thomson *et al*, 1985; Fagg, 1987) and cause impaired cognitive function including working memory performance in healthy humans (Javitt and Zukin, 1991; Krystal *et al*, 1994; Malhotra *et al*, 1996; Jentsch and Roth, 1999).

NMDA receptors are known to interact with the central dopaminergic system (Roberts and Anderson, 1979; Carter *et al*, 1988; Krebs *et al*, 1991; Morari *et al*, 1993; Youngren *et al*, 1993; Tsukada *et al*, 2000b, 2001b). Dopamine (DA) innervation of PFC is considered to be highly responsive to NMDA receptor antagonists (Deutch *et al*, 1987; Bowers and Morton, 1994; Hondo *et al*, 1994; Carlezon and Wise, 1996; Verma and Moghaddam, 1996; Jentsch *et al*, 1997a). The effect of noncompetitive NMDA receptor antagonists on DA metabolism is more enhanced in the mesocorticolimbic dopaminergic system than in the nigrostriatal dopaminergic system (Deutch *et al*, 1987; Rao *et al*, 1990). These modulations of PFC DA may be relevant to the psychotic effects of NMDA receptor antagonists, and these responses are, in part, inhibited by blocking dopaminergic transmission (Murray and Horita, 1978; Fessler *et al*, 1979).

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A very large body of data supports the hypothesis that chronic NMDA receptor antagonism reduces extracellular DA levels, modulates DA neurotransmission in prefrontal cortex (PFC), and alters working memory (for a review, see Jentsch and Roth, 1999). Although data supporting this hypothesis are extensive, they are fragmented with some findings in animals, both *in vivo* and *ex vivo*, and others in humans.

Positron emission tomography (PET) noninvasively measures the neuroanatomical distribution of radiolabeled DA-specific ligands in the living brain. However, the region of interest (ROI) has been limited to the striatum due to the lack of suitable labeled compounds for extrastriatal regions with a low density of DA neuronal terminals (Hall *et al*, 1988; Lidow *et al*. 1989). [^{11}C]NNC112 (Halldin *et al*, 1998) and [^{11}C]FLB457 (Halldin *et al*, 1995) have been developed to assess the extrastriatal neocortical receptor D_1 (D_1R) and D_2 (D_2R), respectively. Recent PET studies have suggested the dysfunction of the extrastriatal dopaminergic system in schizophrenic patients (Okubo *et al*, 1997; Abi-Dargham *et al*, 2002; Suhara *et al*, 2002).

The purpose of the present research is to provide definitive *in vivo* data in subhuman primates (monkeys) using multidisciplinary methodologies that NMDA antagonism by dizocilpine (MK-801) affects all three variables as currently hypothesized. The results obtained provide the basis for further studies of normal brain function and its alterations in stress, mental disease, such as schizophrenia, and drug abuse with ketamine and phencyclidine (PCP).

MATERIALS AND METHODS

Animals, Drugs, and Colocalization

Eight young-adult male rhesus monkeys (*Macaca mulatta*) weighing from 5.5 to 7.0 kg were used in these studies. 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, Hamamatsu Photonics. Magnetic resonance images (MRI) of each monkey under pentobarbital anesthesia were obtained with a Toshiba MRT-50A/II (0.5 T, Toshiba Medical Corporation, Tokyo, Japan) for the purposes of brain colocalization. PET data were collected on a high-resolution PET scanner (Hamamatsu SHR-7700) as described below. PET images were generated by summation of image data from 37 to 64 min after injection. The stereotactic coordinates for PET and MRI were coregistered based on the orbitomeatal (OM) line. A specially designed head holder was used to restrict monkey head movements as described in our publications referred below.

For the acute study, 30 min before injection of [^{11}C]NNC112 or [^{11}C]FLB457, MK-801 in doses of 0 (saline), 0.03, 0.1, and 0.3 mg/kg was administered i.v. at 0900 hours. After 1–2 weeks, for the chronic daily study, the lowest dose of 0.03 mg/kg of MK-801 was given i.m. b.i.d. at 0900 and 1700 hours for 13 days. On the 7th and 14th day 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 in these trained, habituated monkeys with an interval of at least 15 h after the last dose of MK-801. (+)-MK-801 was purchased from RBI (Natick, MA, USA). FLB457 and the precursor of [^{11}C]FLB457 were obtained from ABX (Dresden, Germany). NNC112, the precursor of [^{11}C]NNC112, were the kind gifts of Professor Christer Halldin of the Karolinska Institute, Stockholm, Sweden.

Synthesis of ^{11}C-Labeled Compounds

Carbon-11 (^{11}C) was produced by a $^{14}N(p,\alpha)^{11}C$ nuclear reaction using the cyclotron (HM-18, Sumitomo Heavy Industry, Tokyo, Japan) at Hamamatsu Photonics PET Center. The formed [^{11}C]CO_2 was converted to [^{11}C]methyl iodide via [^{11}C]methane using the PET Trace MeI MicroLab method (GE Medical Systems, Milwaukee, Wis.). [^{11}C]NNC112 was labeled with ^{11}C by N-methylation of its nor-compound with [^{11}C]methyl iodide (Halldin *et al*, 1998). [^{11}C]FLB457 was labeled with ^{11}C by O-methylation of its nor-compound (Halldin *et al*, 1995). The radiochemical and chemical purities of the labeled compounds were greater than 98 and 99%, respectively; their specific radioactivity ranged from 306 to 403 GBq/μmol for [^{11}C]NNC112, and from 348 to 372 GBq/μmol for [^{11}C]FLB457. After chemical analysis for identification and purity, the solution was passed through a 0.22 μm pore filter before i.v. administration to each monkey.

PET Scans in Conscious Monkeys

As described above, data were collected with a high-resolution PET scanner. The transaxial resolution was 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, the trained and habituated animals were fixed by a skull mounted plastic cap to the monkey chair with stereotactic coordinates aligned parallel to the OM line. PET scans of the habituated and trained monkeys were performed in the conscious state (Tsukada *et al*, 1999a, b, 2000a, b, 2001a, b, 2002, 2004) in order to eliminate the effects of anesthetics. 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 frames at 30 s, 12 frames at 1 min, followed by 16 frames at 3 min.

PET Data Analysis

Quantitative time–activity curves of radioactivity in the cerebellum were used as the input function because of its much lower density of DA receptors (Creese *et al*, 1975). Each 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) as per the quantitative analysis of [^{11}C]NNC112 and [^{11}C]FLB457. In the present study, PET measurements were repeated frequently with 1 week intervals in the same monkey. It was not permitted by the Animal Ethical Committee to perform arterial blood sampling each time. Therefore, the reference tissue model

analysis was selected instead of Logan graphic plots, or three-compartment modeling.

Behavioral Tasks

Behavioral task performance was evaluated as described previously (Inoue *et al*, 2004; Tsukada *et al*, 2004). Briefly, in the oculomotor delayed response (ODR) task, after a short intertrial 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 the 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.

In the single-dose acute study, 30 min before the beginning of the task saline or MK-801 in randomized doses of 0.03, 0.1, and 0.3 mg/kg was given i.v. Within 1–2 weeks, the chronic daily bid study was begun. On the 7th and 14th days after the beginning of daily MK-801 administration, behavioral analysis was performed prior to MK-801 administration. In the single acute and chronic bid MK-801 treatments, the delay period between cue presentation and saccade timing was fixed at 6 s.

Microdialysis Analysis

A guide cannula was previously implanted 35 mm anterior to the intrameatal line and lateral 10 mm from the midline (A: 35, L: 10) according to the individual MR images. A microdialysis probe with a membrane region 250 μm in diameter and 3 mm in length (Eicom A-I-8-03, Eicom, Tokyo, Kyoto) 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, 75 μl samples were collected every 15 min, and DA and glutamate contents were measured by HPLC systems (HTEC-500 and DTA-300, Eicom, Kyoto, Japan). These methods have been described previously by Tsukada *et al* (2000a, b).

In the single-dose MK-801 study, the mean data obtained from 0 to 120 min before administration of saline or MK-801 were used as 'baseline'. Saline or MK-801 (0.03, 0.1, and 0.3 mg/kg) was administered 120 min after the beginning of sampling. The levels of glutamate and DA in the extracellular fluid (ECF) of PFC were expressed as '% of baseline'. In the chronic study, only 'baseline' data were evaluated for 120 min during PET measurements on the 7th and 14th days.

Statistical Analysis

Results were expressed as means ± SD. Comparisons between the pretreatment 'baseline' and 'MK-801 administration' were carried out using a paired, two-tailed Student's *t*-test. A probability level of less than 5 % ($P < 0.05$) was considered significant.

RESULTS

Figures 1 and 2 illustrate the PET images and radioactivity uptake curves of [^{11}C]NNC112 and [^{11}C]FLB457 in the conscious monkey. High uptake was in the striatum. Medium uptake was in neocortical regions. Low uptake was in the cerebellum. This pattern is typical in normal monkey brain (Figure 1, 2a and b).

Quantitative kinetic analyses revealed that i.v. single-dose administration of MK-801 produced no significant changes in [^{11}C]NNC112 binding to D_1R in any cortical region in any dose (Figure 3a). In contrast, there was a dose-dependent reduction in [^{11}C]FLB457 binding to D_2R in the PFC, but not in the TMC or OCC after doses of 0.03, 0.1, and 0.3 mg/kg of MK-801 (Figure 3b).

The effects of single doses of MK-801 on glutamate and DA extracellular concentrations in PFC ECF are shown in Figure 4. Baseline levels of glutamate and DA were

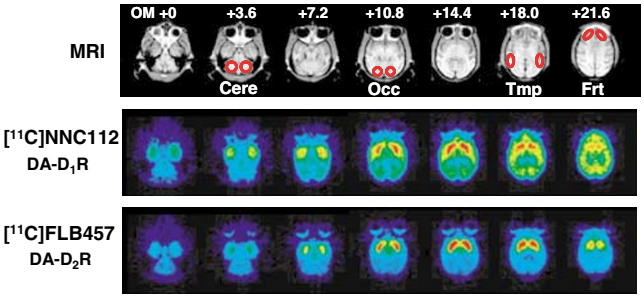

Figure 1 Typical MRI and PET brain images of [^{11}C]NNC112 (a) and [^{11}C]FLB457 in the conscious rhesus monkeys (*Macaca mulatta*). MR images were obtained with a Toshiba MRT-50A/II (0.5 T). PET data were collected on a high-resolution PET scanner (Hamamatsu SHR-7700) with transaxial resolution of 2.6 mm (FWHM) and a center-to-center distance of 3.6 mm. PET images were generated by summation of image data from 37 to 64 min after injection. The stereotactic coordinates of PET and MRI were adjusted based on the orbitomeatal (OM) line. Cere: cerebellum, Occ: occipital cortex. Tmp; temporal cortex, Frt; prefrontal cortex. Regions of interest (ROIs) are identified in the MRIs as illustrated.

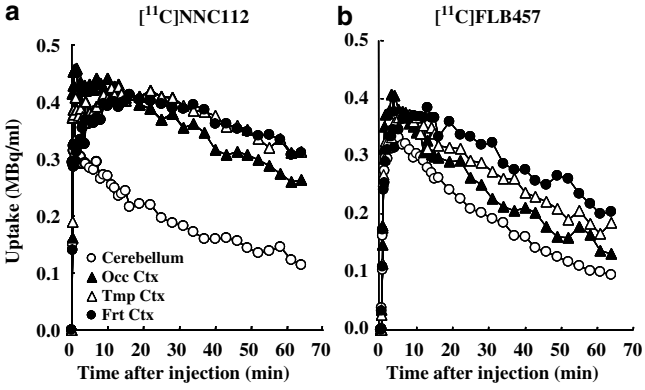

Figure 2 Typical time–activity brain uptake curves of [^{11}C]NNC112 (a) and [^{11}C]FLB457 (b) in a conscious monkey. PET scans were begun immediately after i.v. injection of each tracer. Image data were collected for 64 min. The ROIs were the cerebellum, occipital (Occ Ctx), temporal (Tmp Ctx), and prefrontal cortices (Frt Ctx) selected from the MRI of the same animal. The radioactivity in each ROI is plotted against time after injection.

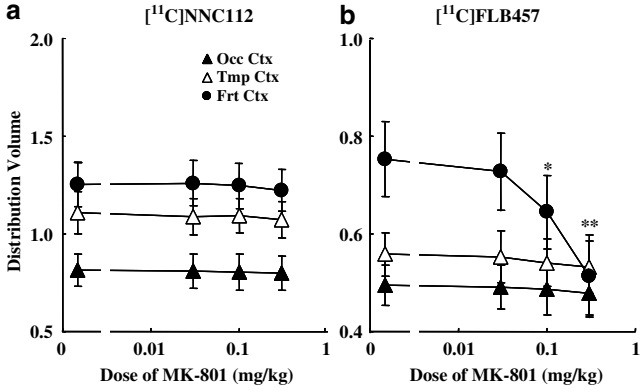

Figure 3 Effects of acute single-dose administration of MK-801 on binding of [^{11}C]NNC112 (a) and [^{11}C]FLB457 (b) in conscious monkey brains. MK-801 (0.03, 0.1, and 0.3 mg/kg) was administered i.v. just before tracer injection. Time–activity curves of radioactivity in the cerebellum and each ROI were fitted to a two-compartment model using the least-square method. The distribution volume ($DV = K_1/k_2$) in each ROI was calculated. Each data point represents the mean and the vertical bars ($\pm$SD) in this and subsequent figures. $N = 8$, *; $P < 0.05$ *vs* Dose = 0, **; $P < 0.01$ *vs* Dose = 0.

1.32 ± 0.25 and 0.52 ± 0.07 fmol/µl, respectively, in the PFC of conscious monkeys. When MK-801 was administered in doses of 0.03 and 0.1 mg/kg, glutamate levels increased in PFC ECF (Figure 3a). In contrast, MK-801 in a large dose of 0.3 mg/kg produced conversely a slight reduction in extracellular glutamate levels in the PFC (Figure 4a). Administration of MK-801 (0.03, 0.1, and 0.3 mg/kg) produced dose-dependent increases in the extracellular DA levels in PFC (Figure 4b).

With saline treatment, there were no significant delay-dependent effects on VGS task performance. However, a delay-dependent reduction in the correct response was observed in ODR task performance, showing 79% accuracy at a 6-s delay period (Figure 5a). Single doses of MK-801 (0.03, 0.1, and 0.3 mg/kg) resulted in a dose-dependent impairment in the ODR task with a similar 6-s delay period.

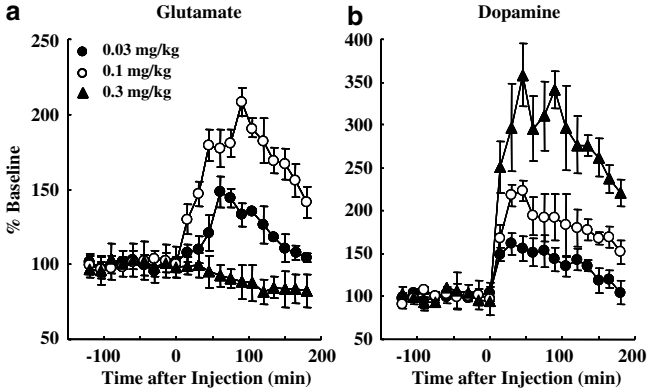

Figure 4 Effects of acute single-dose administration of MK-801 on glutamate (a) and DA (b) in the extracellular fluid (ECF) of prefrontal cortex (PFC) in conscious monkey brains. A microdialysis probe was inserted into the PFC region via a guide cannula. The probe was perfused with a modified Ringer solution at a rate of 5 µl/min. Samples were collected every 15 min. The glutamate and DA concentrations were measured by two separate HPLC systems. The averaged data obtained from 0 to 120 min without any infusion were used as 'baseline' data. MK-801 (0.03, 0.1, and 0.3 mg/kg) was administered 120 min after the beginning of sampling (Time = 0). The glutamate and DA levels were expressed as '% baseline'.

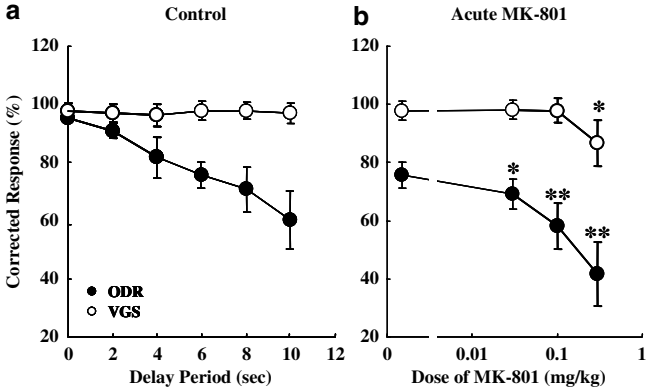

Figure 5 Effects of the central delay period (a) and acute single doses of MK-801 (b) on working memory performance determined by oculomotor delayed response (ODR) and visually guided saccade (VGS) tasks in conscious monkeys. At 30 min before the start of each task, 0.9% NaCl or MK-801 (0.03, 0.1, and 0.3 mg/kg) was administered i.v. In the 'Control' condition (a), the delay period was randomly varied from 0.5 to 10 s. In the 'MK-801' condition, the delay period between cue presentation and saccade timing was fixed for 6 s. *; $P < 0.05$ *vs* Dose = 0, **; $P < 0.01$ *vs* Dose = 0.

There was no impairment on the VGS task in doses of 0.03 and 0.1 mg/kg (Figure 5b). A large dose of MK-801 (0.3 mg/kg) slightly impaired VGS task performance (Figure 5b). With this dose, glutamate concentrations in PFC ECF were decreased (Figure 4a).

During chronic daily bid treatment with MK-801 with a low dose of 0.03 mg/kg, [^{11}C]NNC112 binding to D_1R significantly increased on the 14th day in the PFC, but not in TMC and OCC (Figure 6a). In contrast, [^{11}C]FLB457 binding to D_2R showed no significant changes in any cortical regions on the 7th day. There was a tendency to increase D_2R binding, but this did not reach significance in

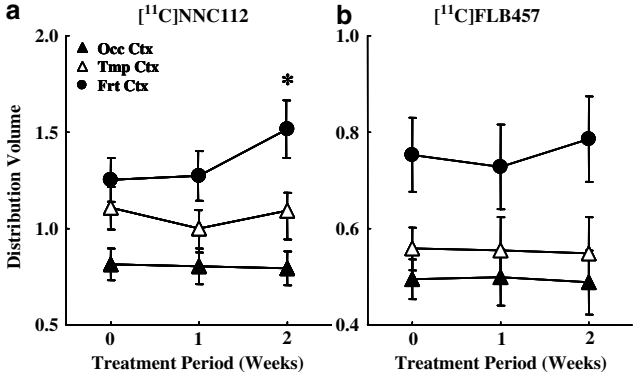

Figure 6 Effects of daily chronic administration of MK-801 on binding of [^{11}C]NNC112 (a) and [^{11}C]FLB457 (b) in conscious monkey brain. MK-801 in a dose of 0.03 mg/kg was given i.m. b.i.d. for 13 days. On the 7th and 14th days, PET scans with [^{11}C]NNC112 and [^{11}C]FLB457 were performed with an interval of at least 15 h after the last dose of MK-801. Distribution volume ($DV = K_1/k_2$) in each ROI was calculated as shown in Figure 1. *; $P < 0.05$ *vs* Dose = 0.

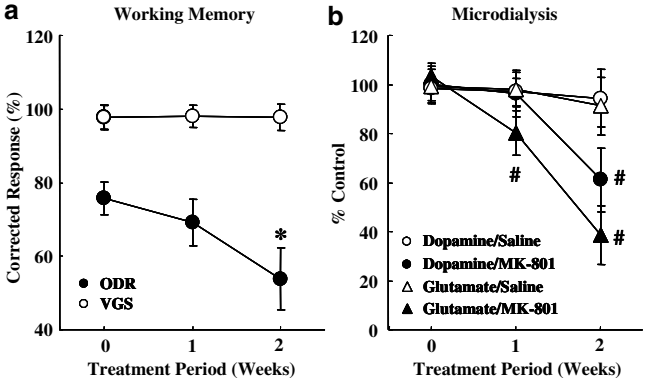

Figure 7 Effects of daily chronic administration of MK-801 on working memory performance (a) and glutamate and DA levels in PFC (b). MK-801 in a dose of 0.03 mg/kg was given i.m. b.i.d. for 13 days. On the 7th and 14th days, working memory task and microdialysis analyses were performed with an interval of at least 15 h after the last dose of MK-801 administration. *; $P < 0.05$ *vs* dose = 0, #; $P < 0.01$ *vs* corresponding saline condition.

PFC on the 14th day in this small group of monkeys (Figure 6b).

On the 14th day with chronic MK-801 administration, there were no significant changes in VGS task performance. In contrast, ODR task performance, with a 6-s delay period, showed marked impairment (Figure 7a).

As shown in Figure 7b, chronic daily bid injections of MK-801 produced a significant reduction in baseline glutamate levels on the 7th day, followed by decreased levels of both DA and glutamate on the 14th day.

To confirm a possible relationship between MK-801-induced impairment of working memory performance and DA receptor function, ODR task performance was plotted against PFC DV of [^{11}C]FLB457 (acute single doses) or [^{11}C]NNC112 (in chronic daily bid doses) in each animal (Figure 8). ODR task performance showed a positive correlation with [^{11}C]FLB457 binding to PFC D_2 receptors after acute MK-801 treatment at 0.1 mg/kg ($r^2 = 0.705$,

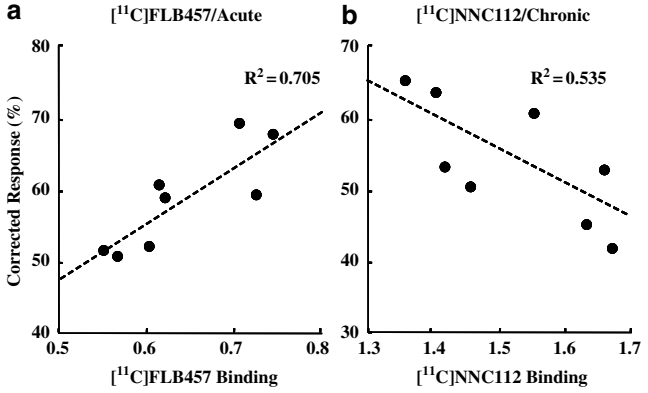

Figure 8 Relationship between MK-801-induced impairment of working memory performance and DA D_2 (a) and D_1 (b) receptor binding in the PFC of conscious monkeys. Working memory performance was evaluated with the ODR task with an acute dose of MK-801 of 0.1 mg/kg (a) and with chronic bid treatments for 13 days in a dose of 0.03 mg/kg. ODR task performance was plotted against distribution volume ($= K_1/k_2$) of [^{11}C]FLB457 (a) or [^{11}C]NNC112 (b) in the PFC of each animal. Note the correlation of coefficients.

Figure 7a); with this dose, VGS task performance was not affected (Figure 5b). In contrast, an inverse correlation between ODR task performance and [^{11}C]NNC112 binding to the PFC D_1 receptors was obtained in the PFC ($r^2 = 0.535$) after daily bid MK-801 treatment for 2 weeks in a dose of 0.03 mg/kg (Figure 8b).

DISCUSSION

The Role of DA/Glutamic Acid and Working Memory

Working memory is impaired when DA is depleted in dorsolateral PFC of monkeys (Brozoski *et al*, 1979). Subsequently, Goldman-Rakic and Brown (1981) showed regional changes of monoamines in aging rhesus monkeys. Goldman-Rakic (1987) summarized the knowledge then available on the circuitry of the PFC and working memory mechanisms. Sawaguchi and Goldman-Rakic (1991) then showed that intracerebral injection of the D_1R antagonist increased the error rate of a delayed response task. Another major advance was the discovery that the predominant mechanism of action of PCP, ketamine, and MK-801 was noncompetitive antagonism of the NMDA receptors of glutamic acid (Lodge *et al*, 1983; Thomson *et al*, 1985; Fagg, 1987). Acute administration of noncompetitive NMDA antagonists preferentially increase the release of DA in the PFC (Doherty *et al*, 1980; Deutch *et al*, 1987; Bowers and Morton, 1994; Hondo *et al*, 1994; Carlezon and Wise, 1996; Verma and Moghaddam, 1996; Jentsch *et al*, 1997a), probably through suppression of cortical NMDA-GABAergic synapses (Grunze *et al*, 1994; Yonezawa *et al*, 1998; Shi and Zhang, 2003) that normally inhibit DA release. It appears to be a paradox that typical psychomotor stimulants such as amphetamine (During *et al*, 1987) and cocaine (Sorg and Kalivas, 1993), noncompetitive NMDA receptor antagonists (Deutch *et al*, 1987; Bowers and Morton, 1994; Hondo *et al*, 1994; Carlezon and Wise, 1996; Verma and Moghaddam, 1996; Jentsch *et al*, 1997a), as

well as acute stress (Murphy *et al*, 1996a, b) all induce impaired working memory performance via hyperdopaminergic neurotransmission. Those data indicate that activation of dopaminergic neurotransmission in the PFC also impairs working memory. The present microdialysis analysis indicates that DA levels in PFC were significantly increased by acute low-dose MK-801, but decreased after chronic treatment. The present ODR task performance results also demonstrate that both hyper (induced by acute MK-801 exposure)- and hypo (by chronic exposure)-DA transmission impairs working memory performance. Taken together, one can conclude that proper functioning of the DA system in PFC critically contributes to working memory. It has been reported that ketamine, a noncompetitive NMDA receptor antagonist, attenuates sensory perception (Oye *et al*, 1992), suggesting that the present data of impaired working memory performance by MK-801 might be produced by a general reduction or distortion of sensory input that affects the performance of any spatial-related task. However, this is unlikely, because the doses of MK-801 used in the present study altered the ODR and not the VGS task, which does not involve a delay.

Morphological, biochemical, and pharmacological studies also have indicated a close interaction between excitatory amino acid and DA afferents in PFC. Dopaminergic and glutamatergic terminals are localized in close opposition to each other on the same postsynaptic pyramidal cell (Smiley *et al*, 1994). NMDA receptors are known to interact with the central dopaminergic system (Roberts and Anderson, 1979; Carter *et al*, 1988; Johnson and Jones, 1990; Krebs *et al*, 1991; Morari *et al*, 1993; Youngren *et al*, 1993; Tsukada *et al*, 2000b, 2001b). Furthermore, NMDA receptor antagonists reduce GABA release. In addition, glutamate release increase locally may stimulate DA release via non-NMDA receptors. Glutamate release may also occur as shifts between burst and nonburst firing of DA neurons. The DA innervation of PFC is considered to be highly responsive to psychomotor stimulants such as amphetamine (During *et al*, 1987), cocaine (Sorg and Kalivas, 1993), as well as NMDA receptor antagonists (Deutch *et al*, 1987; Bowers and Morton, 1994; Hondo *et al*, 1994; Carlezon and Wise, 1996; Verma and Moghaddam, 1996; Jentsch *et al*, 1997a).

Prefrontal Cortical DA Receptor Changes Measured by PET

Previous DA imaging studies with PET have been limited to the striatum due to the lack of suitable labeled compounds for the extrastriatal regions. These have a lower density of DA neuronal terminals compared to the striatum (Hall *et al*, 1988; Lidow *et al*, 1989). Recently, [^{11}C]NNC112 (Halldin *et al*, 1998) and [^{11}C]FLB457 (Halldin *et al*, 1995) have been developed to assess the extrastriatal D_1R and D_2R, respectively, and were used in the present study. The results indicate that the acute administration of single doses of MK-801 produced a dose-dependent reduction in [^{11}C]FLB457 binding to PFC D_2R, but not in the striatum. Perhaps kinetic and distribution issues are involved. One possible explanation for reduced binding in PFC might be competition between [^{11}C]FLB457 and synaptic DA enhanced by NMDA inhibition based upon a conventional 'occupancy' theory. This is unlikely, however, because Okauchi *et al* (2001) previously reported that methamphetamine did not affect [^{11}C]FLB457 binding to extrastriatal D_2R, suggesting the insensitivity of [^{11}C]FLB457 for synaptic DA, probably because of its high affinity to D_2R. Instead of the conventional 'occupancy' theory, another explanation may be a 'rate' theory defined as the dynamics of DA binding to receptors and the synaptic turnover of DA (Tsukada *et al*, 1999a, 2000a, b). Further studies are needed on the modulation of [^{11}C]FLB457 binding to extrastriatal D_2R. In contrast to D_2R, no significant changes in [^{11}C]NNC112 binding to PFC D_1R were detected in the acute study. Although the different responses of both ligands to changes in endogenous DA should be taken into account, these results are consistent with the previous observation that impaired working memory performance by acute administration of noncompetitive antagonists of NMDA receptors is reversed by D_2R antagonists, but not by D_1R antagonists (Verma and Moghaddam, 1996).

Chronic administration of MK-801 in the present study elicited increased [^{11}C]NNC112 binding to PFC D_1R, but no significant change in [^{11}C]FLB457 binding to PFC D_2R. As previously reported, chronic administration of PCP reduces DA transmission in the PFC (Jentsch *et al*, 1997b, c); the present microdialysis data demonstrated lowered DA levels in PFC after repeated exposure to MK-801. Decreased DA neuronal activity might possibly contribute to the upregulation of D_1R binding in PFC. In view of the differences between acute and chronic administration of NMDA antagonist treatment, one needs to consider alternative explanations.

NMDA Antagonist Abuse, Schizophrenia, and Stress

PCP has long been recognized as a useful drug model of schizophrenia (Luby *et al*, 1959, 1962; Snyder, 1980; Javitt and Zukin, 1991; Jentsch and Roth, 1999; Domino *et al*, 2004). The subsequent use of PCP and its shorter acting congener, ketamine, by substance abusers has made the former more difficult to study due to its DEA scheduled status. Chronic use of PCP produces robust and enduring cognitive deficits in humans (Cosgrove and Newell, 1991). Similarly, even single doses of ketamine produce reversible deficits in human cognition (Malhotra *et al*, 1996). These substances produce reduction of frontal cerebral blood flow reminiscent of schizophrenia (Weinberger *et al*, 1986). The extensive reviews by Bunney *et al* (1995), Meador-Woodruff and Kleinman (2002), and Krystal *et al* (2004) document the relationships between glutamate, NMDA antagonists, and schizophrenia. Krystal *et al* (1994) have been especially active in using subanesthetic doses of ketamine in normal volunteers as a drug model of schizophrenia. It is of interest that the effect of noncompetitive NMDA receptor antagonists on DA metabolism is greater in the mesocorticolimbic dopaminergic system than in the nigrostriatal dopaminergic system (Deutch *et al*, 1987; Rao *et al*, 1990). These modulations of PFC DA system are relevant to the psychotic effects of NMDA receptor antagonists. Some of these responses are, in part, inhibited by blocking dopaminergic transmission (Murray and Horita, 1978; Fessler *et al*, 1979). The role of the antagonistic effects of NMDA receptors has been of interest because of hypofunction of glutamatergic neurotransmission in the PFC (Carlsson *et al*, 1997), and its

relationship to dopaminergic dysfunction (Robbins, 1990; Grace, 1991; Deutch, 1992), which has been hypothesized in schizophrenic patients. Recent PET studies have supported dysfunction of the extrastriatal dopaminergic system in schizophrenic patients (Okubo *et al*, 1997; Abi-Dargham *et al*, 2002; Suhara *et al*, 2002). The Okubo *et al* (1997) study demonstrated decreased D_1R binding in PFC assayed with [^{11}C]SCH23390, while the Abi-Dargham *et al* (2002) study observed increased binding measured with [^{11}C]NNC112. The reasons for this discrepancy remained unclear until now, although the latter report correctly suggested the upregulation of PFC D_1R binding induced by hypodopaminergic neuronal transmission. In [^{11}C]FLB457 binding to D_2R, Suhara *et al* (2002) reported decreased binding in the anterior cingulated cortex of schizophrenic patients, but not in PFC. The present results did not detect any changes in [^{11}C]FLB457 binding to D_2R, even in the anterior cingulate cortex (data not shown), perhaps due to the relatively short duration of chronic MK-801 treatment.

The PFC dopaminergic system is also vulnerable to stress. Chronic stress produces a marked reduction of DA transmission concomitant with an increase in D_1R receptor density, resulting in working memory impairment. Both deficits in acquisition and maintenance of a novel short-term memory occur in rats (Mizoguchi *et al*, 2000). These results suggest that the neuronal machinery by which noncompetitive NMDA receptor antagonists first activate DA transmission contributes to the development of long-term inhibition of dopaminergic neurons, resulting in cognitive deficits that occur after repeated administration of NMDA antagonists.

In conclusion, the present results demonstrate that either acute single dose or chronic daily doses of MK-801, which produce NMDA receptor inhibition, impair working memory performance with different levels of dopaminergic modulation as reflected by alterations in D_1R and D_2R binding in the PFC of conscious monkeys. These results suggest that proper functioning of the DA system in PFC is important for working memory-related mechanisms. Changes (either hyper- or hypoactivation) in the dopaminergic neuronal system in PFC probably contribute to the cognitive impairment observed in patients with neuropsychiatric diseases such as schizophrenia.

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