Clozapine, But Not Haloperidol, Prevents the Functional Hyperactivity of N-Methyl-D-Aspartate Receptors in Rat Cortical Neurons Induced by Subchronic Administration of Phencyclidine

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ABSTRACT
Repeated exposure of rats to the psychotomimetic drug phencyclidine (PCP) markedly increased the response of prefrontal cortical neurons to the glutamate agonist N-methyl-D-aspartate (NMDA) relative to agonist a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid. Moreover, acute challenge by PCP produced a significantly reduced block of NMDA-induced current. In addition, the subchronic administration of PCP reduced significantly the paired-pulse facilitation, accompanied by a significant increase of excitatory postsynaptic current variance. These results suggest that repeated exposure to PCP increased evoked release of excitatory amino acids. The enhanced release of excitatory amino acids evoked by NMDA could explain, at least partly, a hypersensitive response to NMDA and a reduced blockade of the NMDA responses by a PCP challenge in rats exposed repeatedly to PCP. Pretreatment with the atypical antipsychotic drug clozapine, but not the typical antipsychotic drug haloperidol, attenuates the repeated PCP-induced effect. Our results support the hypothesis that clozapine may facilitate NMDA receptor-mediated neurotransmission to improve schizophrenic-negative symptoms and cognitive dysfunction. This novel approach is useful for evaluating the cellular mechanisms of action of atypical antipsychotic drugs.

Phencyclidine [PCP, a noncompetitive antagonist of N-methyl-D-aspartate (NMDA) receptors]-induced psychotomimetic state has been suggested to be the best pharmacological model of schizophrenia (Deutsch et al., 1989; Carlsson and Carlsson, 1990; Wachtel and Turski, 1990; Javitt and Zukin, 1991; Olney and Farber, 1995; Jentsch et al., 1997a,b). These observations form the foundation for the glutamate hypothesis of schizophrenia which highlights NMDA receptor hypofunction, in addition to dysfunction of dopamine receptors, as a key mechanism underlying major aspects of schizophrenia, particularly cognitive dysfunction (Carlson and Carlson, 1990; Javitt and Zukin, 1991; Jentsch et al., 1997a,b; Olney and Farber, 1995).

The prefrontal cortex has been implicated in the pathophysiology of schizophrenia (Goldman-Rakic and Selemon, 1997). Cognitive deficit, a core feature of schizophrenia, has been observed, in particular, in long-term PCP abusers (Cosgrove and Newell, 1991; Javitt and Zukin, 1991). PCP and its congener ketamine also exacerbate preexisting symptoms in schizophrenics (Pearlson, 1981; Javitt and Zukin, 1991; Krystal et al., 1994; Malhotra et al., 1996). PCP and other noncompetitive NMDA receptor antagonists, such as ketamine and dizocilpine (MK-801), induce an increase in locomotor activity and stereotyped behavior (Schmidt, 1994; Sturgeon et al., 1982) and impair learning, memory, and cognition in mice, rats, and monkeys (Danysh et al., 1988; Alessandri et al., 1989; Boyce et al., 1991; Verma and Moghaddam, 1996; Jentsch et al., 1997a,b). The chronic PCP-induced cognitive dysfunction could be improved by the atypical antipsychotic drug (APD) clozapine (Jentsch et al., 1997a). More...
over, clozapine, but not the typical APD haloperidol, blunts NMDA antagonist-induced psychosis (Lahti et al., 1995; Malhotra et al., 1997). Consistent with these observations, clozapine, olanzapine, and M100907 [a purported atypical APD and the selective serotonin 5-hydroxytryptamine type 2A receptor antagonist (Kehne et al., 1996)], but not haloperidol or raclopride, facilitate NMDA receptor-mediated transmission (Arvanov et al., 1997; Arvanov and Wang, 1997) and prevent acute PCP-induced blockade of NMDA responses in pyramidal cells of the rat medial prefrontal cortex (mPFC; Wang and Liang, 1998a,b). The aim of this present study was to determine whether repeated exposure of rats to PCP alters NMDA receptor-mediated transmission in cortical slices from control and PCP-treated rats and whether clozapine and haloperidol could prevent the subchronic PCP-induced effect.

**Materials and Methods**

**Drug Administration.** Various groups of male Sprague-Dawley rats (60–250 g) received the following treatment: 1) vehicle, 1 week/48 to 60 h (1 ml/kg i.p., b.i.d. for 1 week, 48- to 60-h withdrawal, n = 15); 2) PCP, 1 week/48 to 60 h (2 mg/kg i.p., b.i.d. for 1 week, 48- to 60-h withdrawal, n = 18); 3) PCP 1 week/1 week (2 mg/kg i.p., b.i.d. for 1 week, 1-week withdrawal, n = 6); 4) PCP, 1 week/0.5 h (2 mg/kg i.p., b.i.d. for 1 week, 0.5-h withdrawal, n = 6); and 5) PCP, single 48 h (single i.p. injection of PCP 5 mg/kg, 48-h withdrawal, n = 6). In case of cotreatment with haloperidol (0.5 mg/kg daily, 2 weeks, n = 6) or clozapine (25 mg/kg daily, 2 weeks, n = 6), rats received i.p. injections of APD for 1 week, then APD plus PCP (2 mg/kg b.i.d.) for the following week, and were sacrificed 48 to 60 h after the last PCP injection. The dose of PCP was selected based on those reported in the literature (Verebey et al., 1981; Sturgeon et al., 1982; Jentsch et al., 1987a,b; Sams-Dodd, 1988) and our observation of marked increase in locomotor activity and stereotype induced by PCP. As has been reported previously (Manallack et al., 1989), there was an apparent tolerance in the aforementioned behavioral effects produced by repeated PCP injection. The doses of clozapine and haloperidol were selected because we have previously demonstrated that subchronic treatment of rats with either clozapine or haloperidol at these doses produced a “depolarization block” of spontaneously active dopamine neurons in the midbrain (White and Wang, 1983a,b).

**Preparation of Slices of the mPFC.** The procedures for preparation of rat mPFC brain slices have been described previously (Yang et al., 1996; Arvanov and Wang, 1997; Arvanov et al., 1997; Wang and Liang, 1998a). Briefly, rats were decapitated under halothane anesthesia and their brains were removed and cooled in ice-cold artificial cerebrospinal fluid (ACSF). The coronal (transverse) slices of mPFC (450-μm thick) were cut in ice-cold ACSF containing 117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 25 mM NaHCO₃, 1.2 mM NaH₂PO₄, and 11 mM d-glucose, aerated with 95% O₂/5% CO₂ (pH 7.4). The brain slices were kept in ACSF at room temperature for at least 1 h to allow for recovery. A single slice was then transferred to a recording chamber (32°C) where it was kept submerged in-between two nylon nets. The chamber was continuously perfused with ACSF at a constant rate of 2 ml/min.

The brain slice offers a number of advantages in conducting the proposed studies. For example, compared to in vivo preparations, stable intracellular or whole-cell recording and pharmacological manipulations can be readily performed in brain slice preparations, which permit us to study the cellular and molecular mechanisms in detail. Compared with the tissue culture system or the acutely dissociated neuron, synaptic circuitry is relatively intact and unaltered in slice preparations.

**Intracellular Recording and Single-Electrode Voltage-Clamp.** Standard intracellular and single-electrode voltage-clamp recording techniques were used to record pyramidal cells in layers V and VI of the mPFC in slice preparations as described previously (Arvanov and Wang, 1997, 1998; Arvanov et al., 1997; Wang and Liang, 1998a). Intracellular recordings were performed using 4 M potassium acetate- or 3 M KCl-filled microelectrodes (tip resistance, 60–90 MΩ) with an Axoclamp 2B (Axon Instruments, Burlingame, CA) amplifier. In current-clamp mode, the bridge balance was continuously monitored and adjusted as necessary. Single-electrode voltage clamp was achieved under discontinuous mode at a sampling rate of 5 to 6.2 KHz (30% duty cycle), a gain of 2.5 to 5 nA/mV. The efficacy of voltage clamp, electrode “settling time,” and input capacitance neutralization at the head stage were continuously monitored on an oscilloscope. Current and voltage records were acquired using the software pClamp 6 (Axon Instruments), filtered at 1 KHz, and analyzed off-line. Voltage and current signals were also recorded on a Gould (Cleveland, OH) Easy Graph Thermal Recorder (TA 240) and two-channel video tape recorder (Instrutech VR-10B Digital Data Recorder, Elmont, NY).

The problems (e.g., space clamping) associated with this method in neurons with extended processes have been discussed elsewhere (Finkel and Redman, 1985). As it has been pointed out, these problems faced during single-electrode voltage clamp may be less acute when dealing with the relative changes following drug application (Madison et al., 1987; Schweitzer et al., 1993).

The electrophysiological criteria we used for distinguishing presumed pyramidal versus nonpyramidal cells have been described previously (Yang et al., 1996; Arvanov et al., 1997; Arvanov and Wang, 1998). In general, the pyramidal cells exhibit a longer spike duration (<1 ms at half-maximum spike amplitude) than that of interneurons and show pronounced spike-frequency adaptation in response to constant-current depolarizing pulses. In contrast, interneurons exhibit a brief duration of their action potentials and lack pronounced spike-frequency adaptation. In each neuron recorded, the membrane properties (e.g., see Table 2) were determined in the normal ACSF using the current-clamp mode of Axoclamp-2B amplifier. Tetrodotoxin (0.5 μM, to block action potentials) and glycine (1 μM, to maximize NMDA induced current) were then included in the perfusion medium, and single-electrode voltage clamp (Vh = −60 mV) was used to study NMDA- and AMPA-induced current responses.

APDs were added to the perfusing ACSF. NMDA was applied by placing a 10-μl drop of 1 or 2 mM solution (with 1:100 dilution factor) on a marked spot in the inflow channel (Rainnie et al., 1994; Zheng and Gallagher, 1995; Holmes et al., 1996; Arvanov et al., 1997; Arvanov and Wang, 1998; Wang and Liang, 1998a). AMPA was applied by placing a 10-μl drop of 0.5 or 1 mM solution (with 1:100 dilution factor) on a marked spot in the inflow channel. Repeated microdrop application of NMDA to the same pyramidal cell with an interapplication interval of approximately 15 min produced a consistent inward current, although the baseline current caused by NMDA may vary from cell to cell. Peak amplitude of responses to sequential applications of two concentrations of NMDA and AMPA was examined and response ratios were determined for each neuron recorded.

We have examined the effect of perfusion of NMDA on pyramidal cells of the mPFC. Bath application of 10 μM NMDA for 3 min evoked a gradual increase of inward current. It often took 3 to 4 min to reach the plateau; it is difficult to obtain a true maximum due to excitotoxicity, desensitization, and/or incomplete equilibrium. Moreover, it required 25- to 30-min interapplication intervals to avoid an obvious desensitization and maintain a stable baseline. Thus, it is rather impractical to study the drug effect on NMDA current using this method. We have also tried the micropressure injection of NMDA. It is much more difficult to estimate the actual concentration of drug and to maintain a stable baseline by using the technique of micropressure injection. Whereas applying negative pressure to the pipette causes dilution of the drug solution in the pipette, without
using retaining pressure, NMDA slowly diffuses out of the pipette and causes receptor desensitization.

Excitatory postsynaptic currents (EPSCs) were elicited by passing two identical rectangular current trials (0.05-msec pulse width, 15–25-μA pulse strength, 40 ms of the interpulse interval) between the tips of a bipolar stainless steel electrode placed in the medial part of the forceps minor, about 1 mm from the recording site. EPSCs were recorded by the electrodes filled with 2 M CsCl plus 25 mM QX 314 (lidocaine N-ethyl bromide quaternary salt, tip resistance 30–50 MΩ) to improve the space clamp and to block voltage-activated Na⁺ and K⁺ channels. Under these conditions, the membrane resistance was usually increased by 30 to 50% (Wuarin et al., 1992). Sixty response traces per epoch were collected and statistical analyzed using pClamp-6 Spike Data Analyses Systems (Arvanov and Wang, 1998).

**Data Analysis.** The results were presented as mean ± S.E.M. paired t test, Student’s t test, ANOVA, and least-significant difference post hoc comparison were used; 0.01 and 0.05 were selected for testing the level of significance.

**Drugs.** The compounds (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propanic acid (AMPA), NMDA, QX 314 and tetrodotoxin were all purchased from Research Biochemicals International (Natick, MA). PCP, clozapine, and haloperidol were generous gifts from National Institute of Drug Abuse, Sandoz (Hanover, NJ), and McNeil Laboratories (Fort Washington, PA), respectively.

**Results**

**Ratios of NMDA/AMPA.** To avoid a marked variation of NMDA responses evoked in different slices, we have adapted the techniques of Beart and Lodge (1990) with a modification. We have examined the ratios of peak currents induced by sequential applications of two concentrations of NMDA and AMPA in each neuron (Table 1; Fig. 1). The concentrations of NMDA (10 and 20 μM) and AMPA (5 and 10 μM) were chosen based on previous experiments (Arvanov et al., 1997; Arvanov and Wang, 1998; Wang and Liang, 1998a) and because they produced a submaximal response. AMPA-induced responses were used as a reference because PCP should not interact directly with the AMPA subtype of glutamate receptors. Indeed, this is supported by the fact that the AMPA/AMPAs ratio was unchanged in all PCP-treated groups, i.e., the ratios were not significantly different from that of the vehicle control group (Table 1). This normalization of data would minimize daily variations and differential sensitivities among cells.

**Effect of Subchronic PCP Treatment on Ratios of NMDA/AMPA and PCP Challenge.** Among groups of animals receiving PCP treatment, a statistically significant increase of NMDA20/AMPA10 and NMDA20/AMPA5 ratios was found in rats receiving 1-week repetitive treatments with PCP (2 mg/kg i.p., b.i.d.) followed by a 48- to 60-h withdrawal period (1 week/48–60 h). Our results suggest that subchronic administration of PCP induced the development of a hyper-sensitive response of the cortical neurons to NMDA. This view is further supported by the finding that an acute challenge of PCP produced a significantly diminished blockade of NMDA-induced inward current in cells recorded from the PCP 1-week/48- to 60-h group (Table 1). The trend for aug-

**Table 1**

Comparison of response ratios for NMDA and AMPA and response to PCP challenge in pyramidal cells of the mPFC from vehicle control and PCP-treated rats

<table>
<thead>
<tr>
<th>Conditions of Treatment</th>
<th>NMDA20/NMDA10</th>
<th>AMPA20/AMPA5</th>
<th>NMDA10/NMDA5</th>
<th>NMDA20/NMDA5</th>
<th>% Blockade of NMDA Response Produced by PCP Challenge</th>
</tr>
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<tbody>
<tr>
<td>Vehicle 1 wk/48–60 h (n = 20)</td>
<td>4.2 ± 0.5</td>
<td>4.3 ± 0.3</td>
<td>1.2 ± 0.5</td>
<td>3.9 ± 1.0</td>
<td>91.1 ± 8.9</td>
</tr>
<tr>
<td>PCP single/48 h (n = 10)</td>
<td>6.3 ± 1.6</td>
<td>4.2 ± 0.5</td>
<td>1.2 ± 0.5</td>
<td>6.6 ± 1.8</td>
<td>76.2 ± 21.1</td>
</tr>
<tr>
<td>PCP 1 wk/0.5 h (n = 5)</td>
<td>4.7 ± 1.0</td>
<td>2.5 ± 0.5</td>
<td>0.6 ± 0.1</td>
<td>2.7 ± 0.4</td>
<td>85.2 ± 18.3</td>
</tr>
<tr>
<td>PCP 1 wk/48–60 h (n = 21)</td>
<td>12.8 ± 2.5*</td>
<td>4.6 ± 0.3</td>
<td>2.3 ± 1.0</td>
<td>11.8 ± 2.3*</td>
<td>59.5 ± 8.8*</td>
</tr>
<tr>
<td>PCP 1 wk/1 wk (n = 10)</td>
<td>7.1 ± 2.2</td>
<td>4.3 ± 0.3</td>
<td>1.5 ± 0.5</td>
<td>8.6 ± 3.4</td>
<td>85.1 ± 7.6</td>
</tr>
</tbody>
</table>

Response ratio was obtained using peak amplitude of current induced by 10 and 20 μM NMDA and 5 and 10 μM AMPA in each neuron voltage clamped at −60 mV. In the PCP single/48-h group, experiments were performed 48 h after a single i.p. injection of PCP, whereas PCP 1 week/0.5 h, PCP 1 week/48 to 60 h, PCP 1 week/1 week represents groups in which experiments were performed at 0.5 h, 48 to 60 h, and 1 week after a 1-week subchronic treatment with PCP, respectively. To calculate % blockade of NMDA responses produced by PCP challenge, we compared NMDA-induced current before and after inclusion of 1 μM PCP to the perfusing ACSF. The response induced by a second application of NMDA after PCP was chosen because the blockade produced by PCP was use-dependent. Values are mean ± S.E.M.; n, number of neurons.

*Significantly different from those of the vehicle controls at p < .05 and p < .01, respectively (ANOVA plus Dunnett’s t test).
mentation of the ratios of NMDA\(_{20}/\text{NMDA}_{10}\) and NMDA\(_{20}/\text{AMPA}_{5}\) still persisted in rats for 1 week after the cessation of subchronic PCP treatment, although the augmented ratios failed to reach statistical significance. This was also true in the case of rats which received only a single PCP injection (5 mg/kg i.p.). These observations suggest that 1) subchronic treatment with PCP is more effective than a single injection of PCP to produce a hypersensitive response of neurons in the mPFC to NMDA, and 2) the subchronic PCP-induced hypersensitive response of mPFC cells to NMDA is relatively long-lasting. Interestingly, in the group receiving repetitive treatment with PCP but only a 0.5-h withdrawal period, the NMDA\(_{20}/\text{NMDA}_{10}\) ratio was not significantly different from that of the vehicle control group. This observation suggests that the residual PCP, which is highly lipophilic (Misra et al., 1979), may have partially blocked the NMDA receptor channel and obscured the hypersensitive response.

**Effect of Subchronic PCP Treatment on Paired-Pulse Facilitation (PPF).** To investigate at the cellular level whether repeated exposure to PCP may affect the presynaptic processes, we examined and compared the PPF of evoked EPSCs in PCP 1-week/48- to 60-h and vehicle 1-week/48- to 60-h groups (Fig. 1C). The PPF (EPSC\(_2/\text{EPSC}_1\)) value for the vehicle control and PCP groups was 1.8 ± 0.1 (n = 21) and 1.1 ± 0.1 (n = 13), respectively. The difference of the PPF values between the two groups was statistically significant (t test, p < .05). This decrease of PPF in PCP-treated rats was associated with an increase (207 ± 61% of controls, p < .05) in EPSC variance (mCV = mean\(^2/\text{SD}\(^2\)). These results suggest that repeated exposure to PCP increases the evoked release of excitatory amino acids (EAAs).

Corresponding to the aforementioned results, there was a significant depolarization of membrane potential and a significant reduction of the spike frequency adaptation and slow after hyperpolarization (sAHP) in PCP 1-week/48- to 60-h rats compared with vehicle controls (Table 2). However, other membrane properties such as neuronal input resistance, spike amplitude, and half-width were not significantly altered. Thus, an increase in the NMDA/AMPA response ratio associated with an increase (207 ± 61% of controls, p < .05) in EPSC variance (mCV = mean\(^2/\text{SD}\(^2\)). These results suggest that repeated exposure to PCP increases the evoked release of excitatory amino acids (EAAs).

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<table>
<thead>
<tr>
<th>Conditions</th>
<th>Resting Membrane Potential</th>
<th>Membrane Resistance</th>
<th>Spike Amplitude</th>
<th>Spike half-width</th>
<th>sAHP(^*)</th>
<th>Spike-Frequency Adaptation(^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n = 23)</td>
<td>−72.7 ± 0.5</td>
<td>54.5 ± 3.5</td>
<td>90.3 ± 1.7</td>
<td>1.2 ± 0.1</td>
<td>5.8 ± 0.6</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>PCP (n = 29)</td>
<td>−69.5 ± 0.6(^*)</td>
<td>46.4 ± 2.8</td>
<td>88.1 ± 1.5</td>
<td>1.1 ± 0.1</td>
<td>3.4 ± 0.3(^*)</td>
<td>5.1 ± 0.5(^*)</td>
</tr>
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</table>

\(^*\) Cells were held at −65 mV; sAHP was obtained by passing 0.1 nA through the recording microelectrode.

\(^\text{a}\) Number of action potentials which were evoked by a depolarizing pulse 0.5 nA, 600 ms; cells were held at −70 mV.

\(^\text{b}\) Significantly different from those of the vehicle control group (p < .05, Student's t tests with Bonferroni's correction).
persistent up-regulation of NMDA receptors and sensitizes the brain to NMDA-induced brain injury in rats. Furthermore, Williams et al. (1992) demonstrated that repeated, but not after 1 day, exposure to (−)/2-amino-5-phosphonopentanoic acid (D-AP5) up-regulated NMDA receptors in cultured cortical neurons of rats. However, it should be pointed out that the results obtained from receptor-binding studies remain controversial. For example, it has been reported that repeated exposure to PCP or MK-801 could either increase the binding capacity $B_{\text{max}}$ of [3H]d-tetra-cylinide (TCP) to cerebral cortical membranes of mice (Saran saari et al., 1993) or decrease $B_{\text{max}}$ of [3H]TCP in the olfactory bulbs of the rat (Quirion et al., 1982). Some studies have demonstrated that even a single injection of PCP or MK-801 was effective in increasing the $B_{\text{max}}$ of NMDA receptors in the rat hippocampus (Gao and Taminga, 1994, 1995). Moreover, Manallack et al. (1989) have shown that subchronic administration of MK-801 in the rat decreases cortical binding of [3H]D-AP5 but not that of [3H]TCP, suggesting that the NMDA primary acceptor site is being independently regulated relative to the PCP sites, at least in response to long-term blockade of the ion channel. The different treatment paradigms (with various NMDA receptor antagonists, different doses, and length of treatment) in different species and various ligands and tissues used for the assay in these studies may contribute to the inconsistent results.

The functional hyperactivity of NMDA receptors might be a homeostatic compensatory response to the prolonged blockade of the NMDA receptor channel by PCP. It is possible that prolonged blockade of NMDA receptors in presynaptic glutamatergic terminals (Connick and Stone, 1988; Montague et al., 1994; Berretta and Jones, 1996; Arvanov and Wang, 1997) and/or in the somadendritic site of the pyramidal cell could have triggered the PCP-induced up-regulation of NMDA receptors.

Reminiscent of up-regulated NMDA receptors induced by subchronic PCP treatment, increased expression of the NMDAR1 receptor subunit and NMDA receptors have been observed in the post-mortem brain of alcoholics and in rats exposed repeatedly to ethanol or pentobarbital (Freund and Anderson, 1996; Chen et al., 1997; Tanaka et al., 1997; Oh et al., 1997). Therefore, the augmented expression of NMDA receptors could represent a compensatory response to the inhibitory action of PCP, ethanol, or pentobarbital on the neuronal NMDA receptors.

Interestingly, the enhanced response of pyramidal cells to NMDA in the PCP-treated animals was accompanied by the decrease of PPF and increase of mCV of the electrically evoked EPSCs. Such a decrease of PPF and increase of mCV is known to be associated with conditions where presynaptic transmitter release is enhanced (Hess et al., 1987; Malinow and Tsien, 1990; Arvanov and Wang, 1997). Thus, our results suggest that repeated exposure to PCP increases presynaptic release of EAAs evoked by electrical stimulation of the forcepts minor, which could be the result of up-regulated NMDA autoreceptors (Connick and Stone, 1988; Montague et al., 1994; Berretta and Jones, 1996; Arvanov et al., 1997). If this is the case, application of NMDA should also enhance the release of EAAs (Montague et al., 1994; Berretta and Jones, 1996; Arvanov et al., 1997) and this could explain, at least in part, the hypersensitive response of mPFC pyramidal cells to NMDA. Furthermore, the released EAAs, in turn, act primarily on postsynaptic non-NMDA receptors and cause membrane depolarization (Arvanov and Wang, 1998; Wang and Liang, 1998a). It is known that the blockade of the NMDA receptor channel by PCP and analogs is voltage-dependent and the recovery of the blockade is strikingly enhanced by continuous exposure to NMDA (Honey et al., 1985; Huttner and Bean, 1988; MacDonald et al., 1987). Therefore, an enhanced release of EAAs by NMDA could also account for a reduced blockade of the NMDA responses by a PCP challenge in rats receiving repetitive PCP treatment.

Interestingly, clozapine, but not haloperidol, was found to prevent the subchronic PCP-induced functional hyperactivity of NMDA receptors. In consonant with our results, chronic treatment with clozapine significantly decreased MK-801 binding in the mPFC (Terazi et al., 1996; McCoy and Richfield, 1996; Giardino et al., 1997) and decreased the expression of NR-2C subunits of the NMDA receptor (Riva et al., 1997). This decrease of the expression of NMDA receptors might be a compensatory reaction to the facilitating effect of clozapine on NMDA receptor-mediated transmission (Arvanov et al., 1997); this effect may contribute to the ability of clozapine to nullify the functional hyperactivity of NMDA receptors in rats exposed repetitively to PCP.

It should be pointed out, however, that subchronic haloperidol treatment also significantly reduced [3H]MK-801 binding in the mPFC (Terazi et al., 1996). Moreover, our preliminary results showed that subchronic treatment with either clozapine or haloperidol induced a marked hyposensitive response of pyramidal cells in the mPFC to NMDA (Wang et al., 1998). The present results show that there was a tendency for haloperidol to reduce subchronic PCP-induced enhancement of NMDA$_{\text{max}}$/NMDA$_{10}$ and NMDA$_{\text{max}}$/AMPA$_{5}$ ratios, although the reduction of the ratios did not reach statistical significance. Obviously, further studies are needed to elucidate the mechanisms by which clozapine but not haloperidol prevents the hypersensitive responses to NMDA in pyramidal cells of the mPFC in rats that have been treated repeatedly with PCP. Additionally, the action of other putative typical and atypical APDs on PCP-induced effect should also be examined and compared.
We have previously shown that clozapine, olanzapine, and M100907, but not haloperidol or raclopride, prevent acute PCP-induced blockade of NMDA responses in pyramidal cells of the mPFC (Wang and Liang, 1998a,b). In the present study, we have further demonstrated that clozapine but not haloperidol prevents subchronic PCP-induced hypersensitive NMDA responses. Consistent with our findings, it has been demonstrated that clozapine, but not haloperidol, blunts NMDA antagonist-induced psychosis (Lahti et al., 1997; Malhotra et al., 1997) and that clozapine effectively ameliorates subchronic PCP-induced impairment of the cognitive performance in monkeys (Dentsch et al., 1997a).

In summary, repeated administration of PCP and electro-physiological detection of the ratio of NMDA/AMPA responses in the cortical slices may be useful for exploring the cellular mechanisms by which PCP produces its psychotomie effect, particularly cognitive dysfunction. With additional studies, this may prove to be a new electrophysiological model for screening and evaluating therapeutic agents targeted for neurological and psychiatric disorders associated with cognitive impairment.

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In summary, repeated administration of PCP and electro-physiological detection of the ratio of NMDA/AMPA responses in the cortical slices may be useful for exploring the cellular mechanisms by which PCP produces its psychotomie effect, particularly cognitive dysfunction. With additional studies, this may prove to be a new electrophysiological model for screening and evaluating therapeutic agents targeted for neurological and psychiatric disorders associated with cognitive impairment.

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