Aberrant regulation of NMDA receptors by dopamine D_4 signaling in rats after phencyclidine exposure

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Dopaminergic dysfunction in the prefrontal cortex (PFC) has been implicated in the pathophysiology of schizophrenia. On the other hand, administration of the NMDAR antagonist phencyclidine (PCP) impairs PFC functions and induces a broad range of schizophrenic-like symptoms, thus has been widely used as an animal model for schizophrenia. This study sought to determine the mechanism by which PCP may alter the dopaminergic functions in PFC. In control rats, activation of dopamine D_4 receptors produced a significant suppression of NMDA receptor transmission in PFC pyramidal neurons, which was dependent on the inhibition of active CaMKII. However, in PCP-treated rats, the D_4 modulation of NMDA receptors was significantly impaired, with the concomitant loss of D_4 regulation of CaMKII activity. In contrast, the D_4 modulation of voltage-dependent Ca^{2+} channels was intact following PCP administration. Furthermore, treatment with the antipsychotic drug clozapine restored the D_4 regulation of NMDA receptors in PCP-treated rats. These findings suggest that the selective disruption of the interaction between D_4 and NMDA receptors in the PCP model, which is attributable to the impaired D_4-mediated downstream signaling, may contribute to the aberrant PFC neuronal activity in schizophrenia.
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Introduction

Schizophrenia is a complex neuropsychiatric disease that is manifested in the form of abnormal mental functions and disturbed behavior. Converging evidence has implicated that the prefrontal cortex (PFC) is a major locus of dysfunction in schizophrenia (Weinberger et al., 1986; Andreasen et al., 1997). Schizophrenics often exhibit deficits in cognitive tasks that are sensitive to the functional integrity of PFC (Goldman-Rakic, 1994). Despite the unclear etiology of schizophrenia, a neurobiological hypothesis of schizophrenia, which includes dysfunctions within several neurotransmitter systems in PFC, is supported by the effective pharmacological therapies that have been developed for the treatment of schizophrenia (Sawa and Snyder, 2002).

Most of the antipsychotic drugs act on the dopamine system (Carlsson, 1974; Seeman et al., 1976; Creese et al., 1976). Their significant efficacy forms the corner stone for the "dopamine hypothesis of schizophrenia" (Carlsson, 1988; Davis et al., 1991). Dopaminergic dysfunction in the PFC has been considered as an important contributing factor to the pathophysiology of schizophrenia (Grace, 1991; Deutch, 1992; Lewis and Lieberman, 2000). Among the five dopamine receptors, D_4 receptor is expressed at a high level in PFC neurons (Mrzljak et al., 1996; Wedzony et al., 2000), and is one of the major targets for the efficient atypical antipsychotic drug clozapine (Van Tol et al., 1991). The key role of D_4 receptors in regulating PFC functions is being revealed by genetic and electrophysiological studies (Rubinstein et al., 1997, 2001, Dulawa et al., 1999; Wang et al., 2002, 2003). However, it is still unclear how the functions of D_4 receptors in PFC may go awry in neuropsychiatric disorders.

The involvement of glutamate in schizophrenia is demonstrated by psychotomimetic properties of several noncompetitive NMDA receptor antagonists, such as phencyclidine (PCP), MK-801 and ketamine (Javitt and Zukin, 1991). Both acute and repeated PCP exposure in humans produces a variety of behaviors resembling schizophrenia, including cognitive deficits, loss of short-term memory and negative symptoms (Luby et al., 1959; Allen and Young, 1978; Snyder, 1980; Krystal et al., 1994; Tamminga, 1998). Furthermore, administration of PCP in schizophrenics dramatically exacerbates their symptoms (Itil et al., 1967; Lahti et al., 1995). Studies in animals have also confirmed the initial observations in human subjects (Handelmann et al., 1987; Verma and Moghaddam, 1996). Thus, systemic administration of PCP represents the most widely utilized model of schizophrenia (Jentsch et al., 1997b, 1998; Adams and Moghaddam, 1998; Moghaddam and Adams, 1998; Jentsch and Roth, 1999). Altered levels of several neurochemicals, such as dopamine, glutamate and parvalbumin, have been found in animals treated with PCP (Deutch et al., 1987; Hondo et al., 1994; Moghaddam et al., 1997; Cochran et al., 2003); however,

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physiological changes in PFC neurons following PCP administration have remained elusive.

Our previous study has shown that the NMDA receptor is one of the key targets of D_4 signaling in PFC pyramidal neurons (Wang et al., 2003). In the present study, we examined the D_4 regulation of NMDAR functions in PFC pyramidal neurons from PCP-treated rats. Our findings indicate that systemic administration of PCP markedly attenuated the effect of D_4 receptors on NMDAR transmission in PFC, due to the impaired D_4 signaling. Insights gained from our results should provide clues for a better understanding of schizophrenia and for the development of novel treatment strategies.

Results

The D_4 modulation of NMDA receptor currents is selectively attenuated in PFC pyramidal neurons from PCP-treated rats

To determine how the interaction between D_4 and NMDA receptors in PFC might be altered in neuropsychiatric disorders, we examined the impact of D_4 receptors on NMDA signaling in pyramidal neurons, the major neuronal population in PFC networks, from rats exposed to PCP.

We first tested the effect of the D_4 receptor agonist PD168077 (Glase et al., 1997; Wang et al., 2002, 2003) on NMDA receptor-mediated currents in PFC neurons isolated from PCP-treated rats. The NMDAR current density (pA/pF) in cells dissociated from PCP-treated rats (91.7 ± 7.0, $n = 30$) was about 70% of that in cells from saline-treated rats (132.5 ± 12.1, $n = 17$). Application of PD168077 (20 μM) produced a significant reduction of NMDAR currents in neurons from saline-injected rats ($17.6 \pm 0.7\%$, $n = 23$, $P < 0.01$, Mann–Whitney U test), similar to what was found before in control rats (Wang et al., 2003). In contrast, PD168077 had little effect on the amplitude of NMDAR currents in neurons from PCP-treated rats ($4.2 \pm 0.8\%$, $n = 32$, $P > 0.05$, Mann–Whitney U test). Representative examples are shown in Figs. 1A–C. The effects of PD168077 on NMDAR currents in PFC neurons from rats with acute (1 h or 1 day) or subchronic (3–5 days) PCP treatment are summarized in Fig. 1D. It is evident that D_4 receptors fail to produce significant modulation of NMDAR channels in neurons from PCP-treated rats.

To assess the impact of PCP on NMDAR transmission, we first compared the ratio of NMDAR-mediated synaptic currents (NMDAR-EPSCs) to AMPAR-mediated synaptic currents (AMPAR-EPSCs) in saline vs. PCP-treated rats. This measure offers the advantage of being independent of the number of synapses activated. A representative example is shown in Fig. 2A. When similar AMPAR-EPSCs were triggered, the NMDAR-EPSCs had smaller sizes in the cell from the PCP-treated rat. As summarized in Fig. 2B, the ratio of NMDAR-EPSCs to AMPAR-EPSCs was 1.31 ± 0.15 ($n = 13$) in control cells, and it was reduced
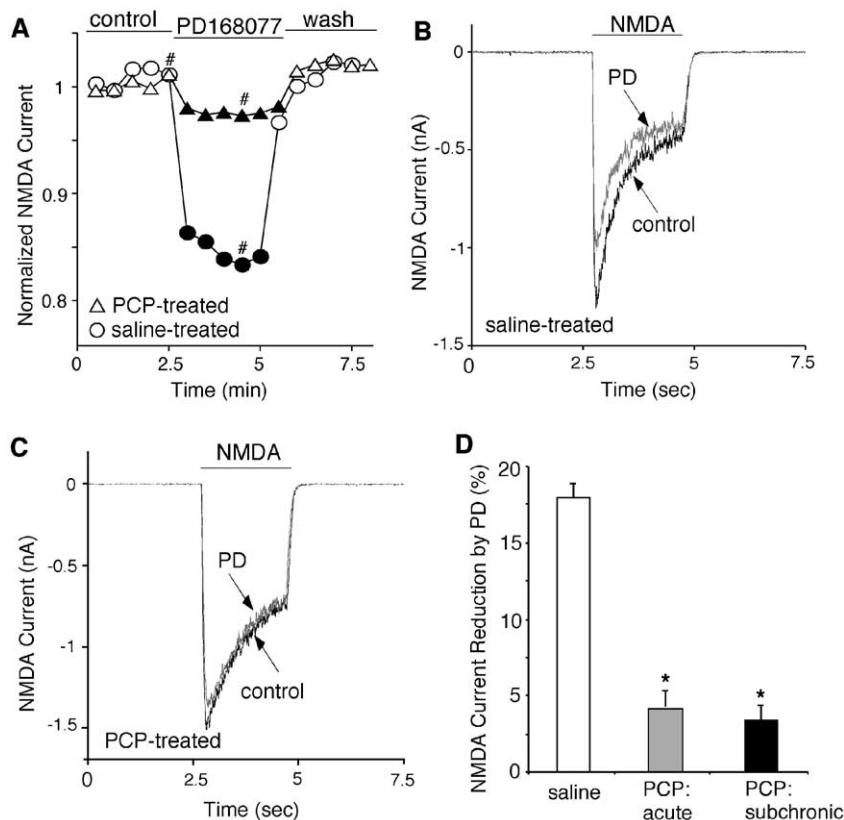

Fig. 1. The D_4 regulation of NMDAR currents was largely abolished in dissociated PFC pyramidal neurons from PCP-treated rats. (A) Plot of peak NMDAR currents as a function of time and drug application in two representative neurons from a saline-treated and a PCP-treated rat, respectively. The ability of PD168077 (40 μM) to reduce NMDAR currents was significantly attenuated in the neuron from a rat injected with PCP (5 mg/kg) for 3 days. (B, C) Representative current traces taken from the records used to construct panel A (at time points denoted by #). (D) Cumulative data (mean ± SEM) showing the percentage reduction of NMDAR currents by PD168077 in rats treated with saline ($n = 23$), acute PCP ($n = 10$) or subchronic PCP ($n = 22$). *$P < 0.01$, t test.

to 0.91 ± 0.11 ($n = 19$) in cells exposed to subchronic PCP. It suggests that systemic administration of PCP caused a $\sim30\%$ decrease of the NMDAR-mediated component of excitatory transmission in PFC pyramidal neurons.

To determine whether the D_4 modulation of NMDAR-mediated synaptic transmission in PFC slices is also affected in the schizophrenia model, we compared the impact of PD168077 on NMDAR-EPSCs in PFC slices from PCP- vs. saline-treated rats. A representative example is shown in Figs. 2C and D. The effect of PD168077 on the amplitude of NMDAR-EPSCs was markedly attenuated in the PFC neuron from a PCP-treated rat, comparing to the cell from a saline-injected rat. As summarized in Fig. 2E, in a sample of PFC pyramidal neurons from rats exposed to acute or subchronic PCP treatment, PD168077 reduced NMDAR-EPSCs to a significantly ($P < 0.01$, t test) smaller extent (acute: $12.1 \pm 2.6\%$, $n = 9$; subchronic: $11.8 \pm 1.8\%$, $n = 15$), comparing to the effect of PD168077 in saline-treated rats ($41.3 \pm 1.8\%$, $n = 8$), suggesting that the D_4 regulation of NMDAR transmission is disturbed by PCP treatment.

To test whether the effect of any agent that acts to reduce NMDA responses is suppressed after PCP treatment due to the lower baseline response to NMDA, we measured the 5-HT$_{1A}$ effect on NMDA receptors in PCP-treated rats. Consistent with our previous finding (Yuen et al., 2005), activation of 5-HT$_{1A}$ receptors with the specific agonist 8-OH-DPAT (20 μM) caused a significant reduction of NMDAR currents in acutely isolated PFC pyramidal neurons (Figs. 3A and B) and a marked decrease of NMDAR-EPSCs in PFC slices (Figs. 3D and E) from saline-treated rats. Interestingly, the effects of 8-OH-DPAT on NMDAR currents and NMDAR-EPSCs were intact in rats treated with subchronic (3 days) PCP (Figs. 3A, B, D, E). In a sample of cells we tested (Fig. 3C), 8-OH-DPAT decreased the amplitude of NMDAR currents by $20.2 \pm 3.9\%$ ($n = 8$) in PCP-treated rats, which was similar to the effect of 8-OH-DPAT in saline-treated rats ($19.3 \pm 2.5\%$, $n = 5$). Moreover, 8-OH-DPAT reduced the amplitude of NMDAR-EPSCs in PCP- vs. saline-treated rats to a similar extent (Fig. 3F, saline: $34.8 \pm 5.2\%$, $n = 5$; PCP: $35.9 \pm 6.5\%$, $n = 6$). It suggests that systemic administration of PCP does not render NMDA receptors refractory to inhibitory modulation.
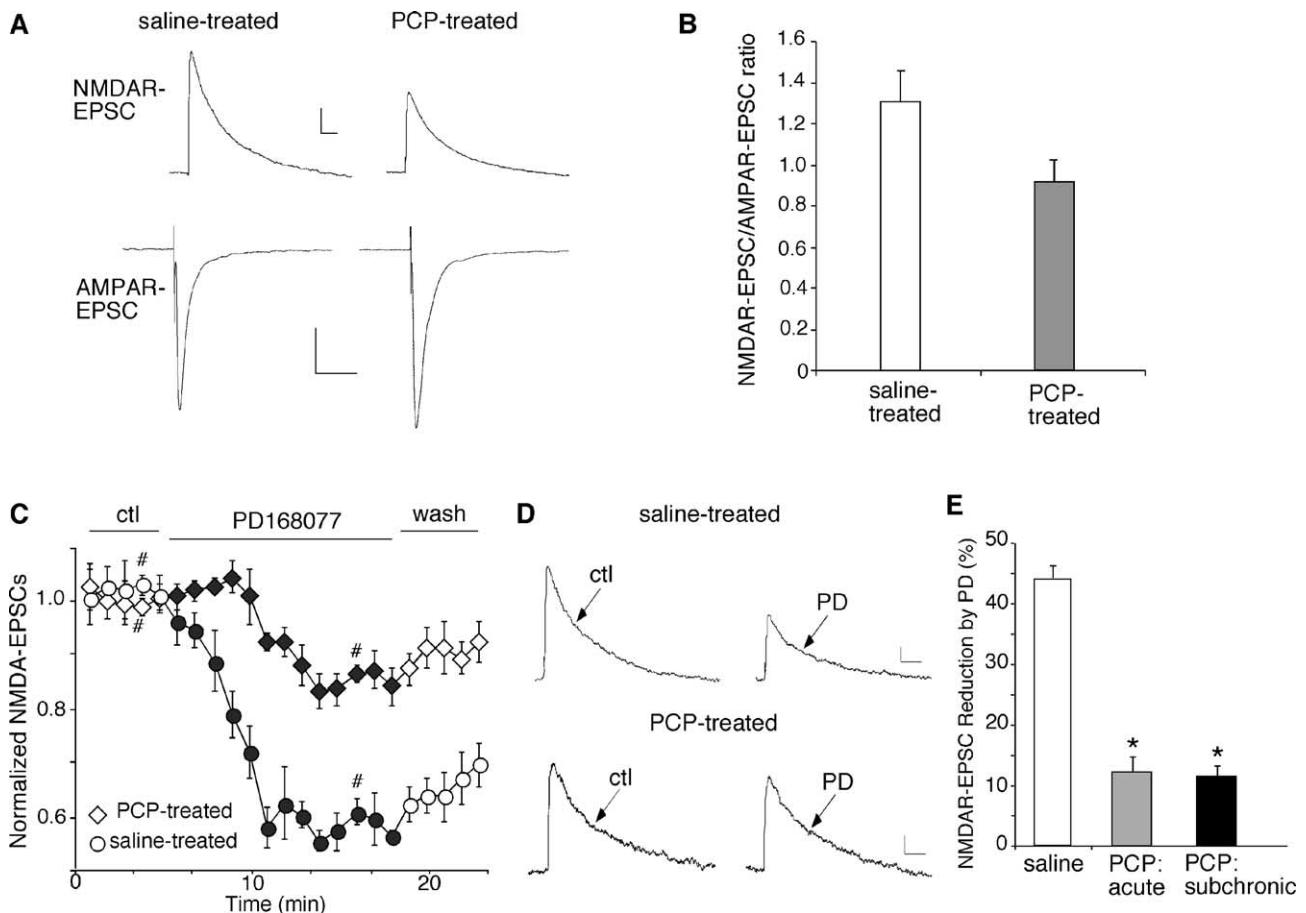

Fig. 2. PCP administration slightly reduced the NMDAR component of excitatory synaptic transmission, but markedly attenuated the D_4 regulation of NMDAR-EPSCs in PFC slices. (A) Current traces (average of 10 raw traces) showing the NMDAR-EPSCs and AMPAR-EPSCs in two PFC pyramidal neurons from rats treated with saline or PCP (5 mg/kg, 3 days). Scale bars: 100 pA, 100 ms (NMDAR-EPSC) or 50 ms (AMPAR-EPSC). (B) Cumulative data (mean $\pm$ SEM) showing the ratio of NMDAR-EPSCs to AMPAR-EPSCs in cells from saline-treated ($n = 13$) or subchronic PCP-treated ($n = 19$) rats. (C) Plot of peak evoked NMDAR-EPSCs as a function of time and agonist (PD168077, 20 μM) application in two representative neurons from rats treated with PCP (5 mg/kg, 3 days) or saline. Each point represents the average peak (mean $\pm$ SEM) of 3 consecutive NMDAR-EPSCs. (D) Representative current traces (average of 3 raw traces) taken from the records used to construct panel C (at time points denoted by #). Scale bars: 25 pA, 100 ms. (E) Cumulative data (mean $\pm$ SEM) showing the percentage reduction of NMDAR-EPSC amplitudes by PD168077 in rats treated with saline ($n = 8$), acute PCP ($n = 9$) or subchronic PCP ($n = 15$). *$P < 0.01$, t test.
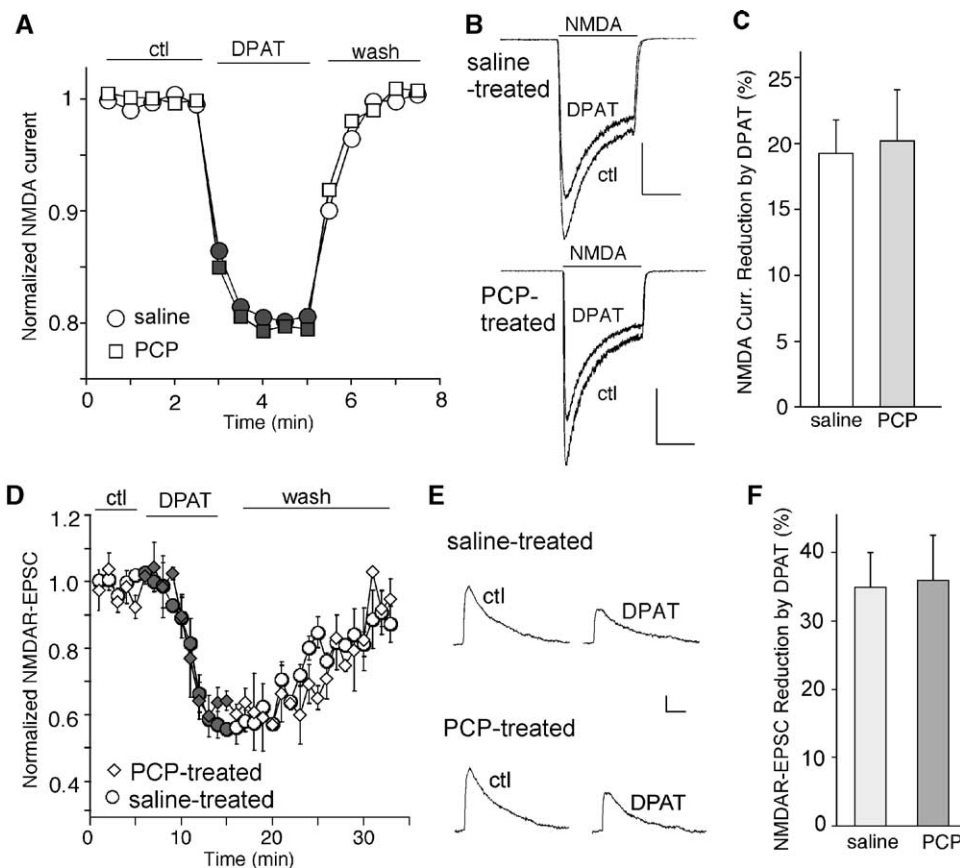
Fig. 3. The 5-HT$_{1A}$ down-regulation of NMDA receptor function was unaffected in PFC pyramidal neurons from PCP-treated rats. (A, D) Plot of peak NMDAR currents (A) or NMDAR-EPSCs (D) as a function of time and drug application in representative neurons from rats treated with PCP (5 mg/kg, 3 days) or saline. The 5-HT$_{1A}$ agonist 8-OH-DPAT (20 μM) reduced NMDAR currents and NMDAR-EPSCs similarly in these neurons. (B, E) Representative current traces taken from the records used to construct panels A (B) or D (E) (at time points denoted by #). Scale bars: 0.5 nA, 1 s (B); 50 pA, 100 ms (E). (C, F) Cumulative data (mean ± SEM) showing the percentage reduction of NMDAR currents (C) or NMDAR-EPSCs (F) by 8-OH-DPAT in rats treated with saline or PCP (3 days).

To further test the specificity of the PCP-induced loss of D$_4$ regulation of NMDA receptors, we also compared the D$_1$ effect on NMDA receptors in PCP- vs. saline-treated rats. As shown in Figs. 4A and B, application of the D$_1$ receptor agonist SKF81297 (10 μM) produced a potent and reversible enhancement of NMDA (500 μM)-evoked currents in PFC pyramidal neurons dissociated from saline-treated rats, similar to our previous results in control rats (Chen et al., 2004). A similar effect of SKF81297 was also found in PCP-treated rats (Figs. 4C and D). In a sample of cells we tested, SKF81297 increased the amplitude of steady-state NMDAR currents by 18.6 ± 1.5% (n = 17) in rats treated with subchronic PCP, which was similar to the effect of SKF81297 in saline-treated rats (19.8 ± 2.2%, n = 14). These results indicate that the D$_1$ receptor-mediated up-regulation of NMDA receptor function is unaltered after PCP treatment.

The D$_4$ modulation of voltage-dependent Ca^{2+} channels is intact in PFC pyramidal neurons from PCP-treated rats

Since the D$_4$ modulation of NMDA receptors is impaired in PFC neurons from PCP-treated rats, we would like to know whether the impairment is due to a selective change in the signaling mechanisms underlying D$_4$ regulation of NMDA receptors or a general dysfunction of D$_4$ receptors. To test this, we examined the effects of PD168077 on voltage-dependent Ca^{2+} channels (VDCCs) in rats

treated with PCP. Ba^{2+} currents through VDCCs were recorded with a voltage ramp protocol (Yan et al., 1997). As shown in Figs. 5A and B, application of PD168077 (40 μM) rapidly and reversibly decreased VDCC currents in PFC pyramidal neurons from rats treated with saline. Meanwhile, PD168077 produced a similar effect on VDCC currents in cells from PCP-treated rats (Figs. 5C and D). In a sample of dissociated PFC neurons we tested, PD168077 reduced the amplitude of VDCC currents by 20.6 ± 5.9% (n = 7) in rats treated with subchronic PCP, which was not significantly different from the effect of PD168077 in saline-treated rats (19.2 ± 2.9%, n = 13, P > 0.05, t test). Since the PD168077 reduction of Ca^{2+} currents can be attributable to the D$_4$R/G protein-mediated direct inhibition of VDCCs (Hille, 1994), the intact effect of PD168077 on VDCCs in PCP-treated rats suggests that PCP administration is unable to affect D$_4$ receptors per se, at least at the early stage. Thus, the selective loss of D$_4$ regulation of NMDA receptors in PCP-treated rats is more likely due to the impaired specific signaling events linking D$_4$ receptors to NMDAR channels.

The aberrant D$_4$ modulation of NMDA receptors in PCP-treated rats is restored by the antipsychotic drug clozapine

To determine whether the loss of D$_4$ modulation of NMDA receptors in PCP-treated rats may be involved in the pathophysi-
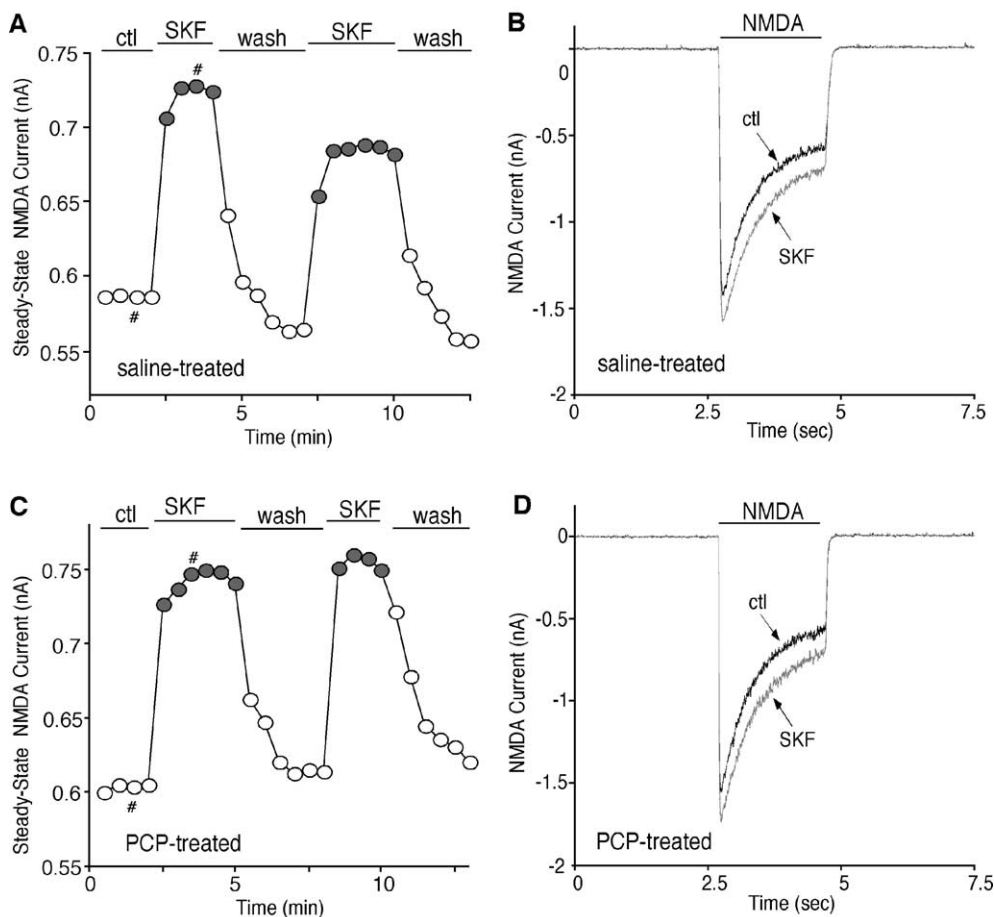
Fig. 4. The D_1 regulation of NMDAR channels in PFC neurons was intact in PCP-treated rats. (A, C) Plot of steady-state NMDAR currents (measured at the end point of 2-s NMDA application) as a function of time and drug application in two representative neurons from rats treated with saline (A) or PCP (C, 5 mg/kg, 3 days). The D_1 agonist SKF81297 (10 μM) produced similar effects on NMDA currents in these cells. (B, D) Representative current traces taken from the records used to construct panels A (B) or C (D) (at time points denoted by #).

ology of schizophrenia, we tested the ability of the "atypical" antipsychotic drug clozapine to restore this D_4 action. Rats were daily injected with clozapine (5 mg/kg) 30 min before PCP (5 mg/kg) administration. This dose of clozapine has been shown to blunt PCP-induced sensorimotor gating deficits (Bakshi et al., 1994) and suppression of social behavior (Steinpreis et al., 1994) in rats. The PD168077 effects on NMDAR currents and NMDAR-EPSCs were compared in rats after subchronic treatment of PCP or PCP plus clozapine. We found that the ability of PD168077 to modulate NMDA receptors was recovered in PCP-rats treated with clozapine. Representative examples are shown in Figs. 6A and B. As summarized in Fig. 6C, PD168077 produced a 16.3 ± 1.0% reduction of NMDAR currents in PFC pyramidal neurons isolated from clozapine-treated rats ($n = 19$), comparable to the effect of PD168077 in control neurons (17.1 ± 1.3%, $n = 12$), but was significantly different from the effect of PD168077 in cells from PCP-treated rats (3.8 ± 1.3%, $n = 7$, $P < 0.01$, t test). Parallel experiments in PFC slices gave similar results (Figs. 6D–F). With subchronic clozapine treatment, PD168077 produced a 37.0 ± 2.9% reduction of NMDAR-EPSCs ($n = 11$), which was significantly bigger than the effect of PD168077 in rats treated with PCP alone (14.4 ± 5.4%, $n = 8$, $P < 0.01$, t test).

The D_4 regulation of CaMKII activity was lost in PCP-treated rats, and was restored by clozapine treatment

Since the electrophysiological data indicate that the D_4 modulation of NMDA receptors is significantly attenuated in PCP-treated rats, we would like to know the potential mechanisms that lead to the altered D_4 action. Our previous study has found that the D_4 depression of NMDAR currents in PFC neurons is dependent on the inhibition of active CaMKII (Wang et al., 2003). We reasoned that one possibility for the aberrant D_4 regulation of NMDA receptors in the schizophrenia model is the inability to regulate CaMKII activity by D_4 receptors. To test this, we compared the effect of PD168077 on CaMKII activation in PFC slices from saline- and PCP-treated rats. CaMKII is autophosphorylated at Thr^{286} when the enzyme is activated in the presence of Ca^{2+}/calmodulin, leading to the appearance of a sustained, Ca^{2+}-independent activity (Miller and Kennedy, 1986). Thus, an anti-Thr^{286}-phosphorylated CaMKII antibody was used to detect activated CaMKII. As shown in Fig. 7, PD168077 potently decreased the activation level of CaMKII in PFC slices from saline-treated rats (32 ± 12% of control after PD168077, $n = 12$, $P < 0.01$, t test, data pooled together), but this effect was almost completely abolished in slices from PCP-treated rats (103 ± 18% of control after
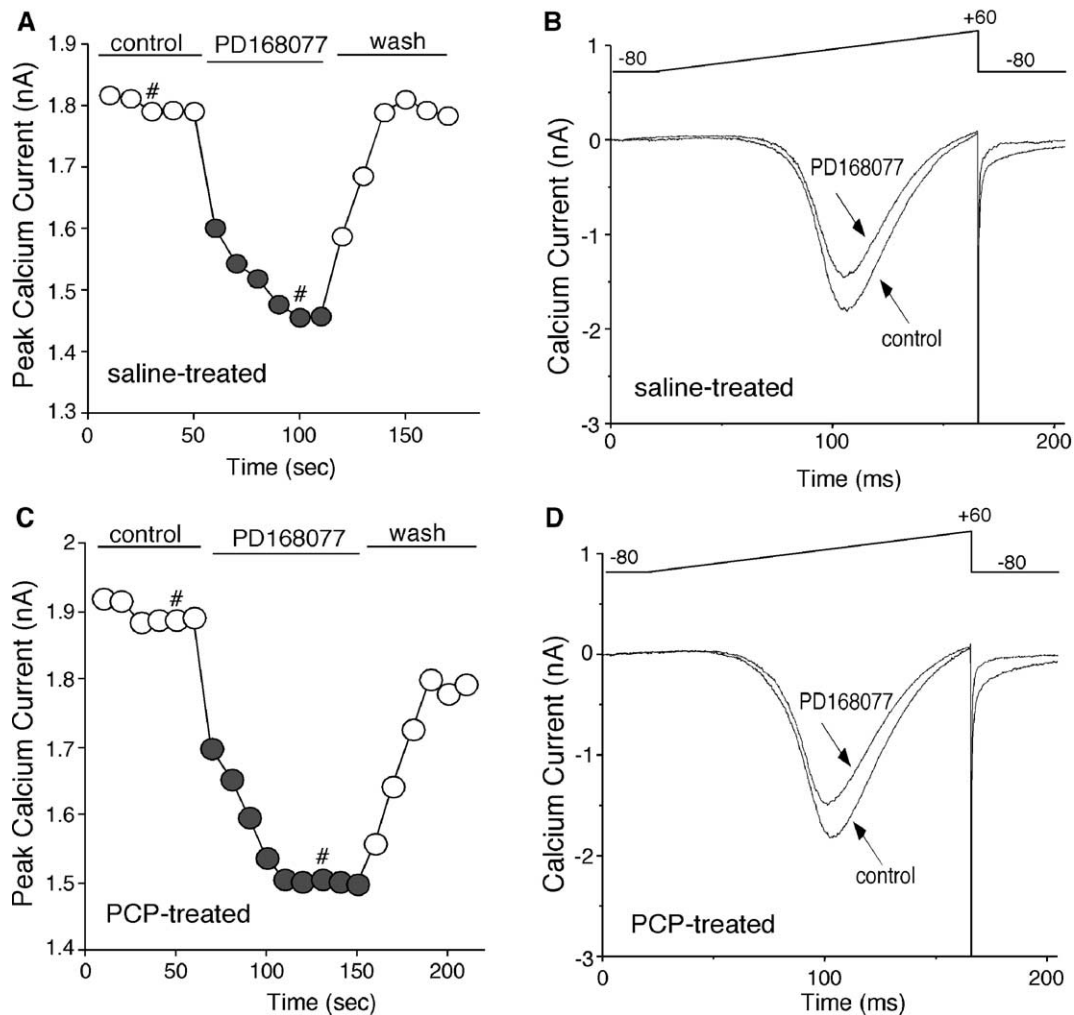
Fig. 5. The D_4 regulation of voltage-dependent Ca^{2+} channels (VDCCs) in PFC neurons was intact in PCP-treated rats. (A, C) Plot of peak Ca^{2+} currents evoked by a voltage ramp protocol as a function of time and drug application in two representative neurons from rats treated with saline (A) or PCP (C, 5 mg/kg, 3 days). PD168077 (20 μM) produced similar effects on VDCCs in these cells. (B, D) Representative current traces taken from the records used to construct panels A (B) or C (D) (at time points denoted by #).

PD168077, $n = 17$, data pooled together). Administration of clozapine in PCP-treated rats significantly restored the ability of PD168077 to regulate CaMKII activity ($42 \pm 11\%$ of control after PD168077, $n = 9$). The total level of CaMKII expression was not significantly changed by PCP treatment. These biochemical results suggest that the D_4-mediated signaling cascade is altered in PCP-treated rats, which may lead to the loss of D_4 modulation of NMDA receptor currents.

Discussion

Behavioral studies have shown that both acute and chronic PCP exposure in humans and animals induces a battery of behavioral symptoms reminiscent of schizophrenia, with the effects of chronic exposure being generally more persistent (Luby et al., 1959; Allen and Young, 1978; Krystal et al., 1994; Jentsch and Roth, 1999). Partial deletion of the gene encoding NMDA receptor NR1 subunit causes several behavioral abnormalities similar to PCP administration (Mohn et al., 1999). Molecular and cellular mechanisms underlying the PCP-induced behavioral

impairment are still unclear. It is proposed that systemic blockade of NMDA receptors triggers a complex polysynaptic network disturbance that is characterized by inactivation of inhibitory neurons and consequent disinhibition of excitatory pathway (Olney et al., 1999). Excessive release of glutamate in PFC has been found with NMDAR blockers (Moghaddam et al., 1997). Increased burst firing in subsets of VTA dopaminergic neurons is also elicited by PCP through an indirect pathway involving PFC glutamatergic neurons innervating VTA (Raja and Guyenet, 1980; Freeman and Bunney, 1984). Moreover, acute administration of PCP profoundly increases the dopamine release in PFC (Deutch et al., 1987; Verma and Moghaddam, 1996; Jentsch et al., 1997a). Therefore, it is thought that the altered NMDA system causes the dysfunction of the dopamine system, leading to the formation of psychotic symptoms. In this study, we sought to determine whether the aberrant dopamine system causes the dysregulation of the NMDA system, thus may contribute to the pathophysiology of schizophrenia.

The enrichment of D_4 receptors in PFC and its high affinity for the atypical antipsychotic drug clozapine have led the speculation that D_4 receptors is highly involved in neuropsychiatric disorders. Earlier studies with subtractive pharmacological methods suggest
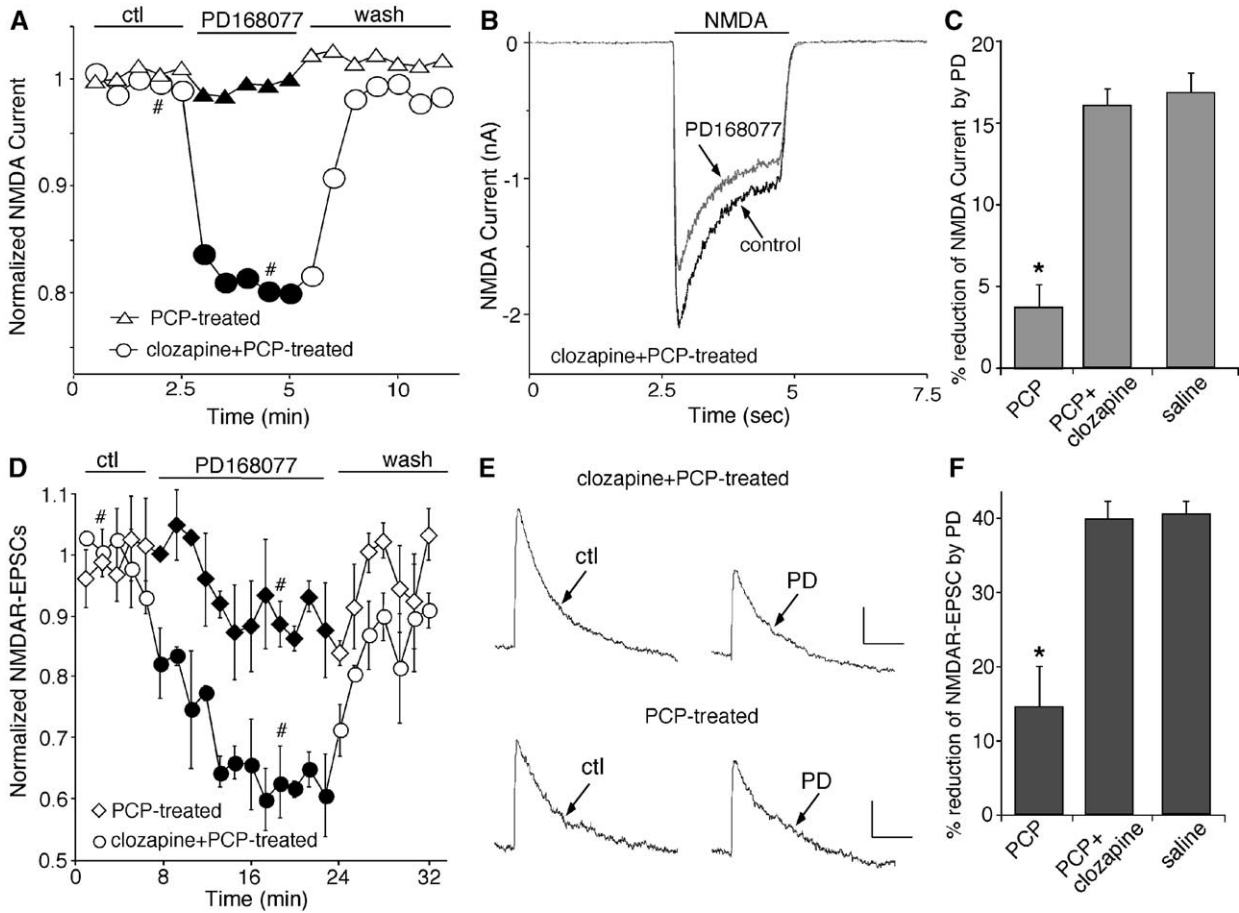
Fig. 6. The D_4 regulation of NMDA receptors in PCP-treated rats was restored by clozapine treatment. (A) Plot of peak NMDAR currents as a function of time and drug (PD168077, 40 μM) application in two representative neurons from rats treated with PCP alone (5 mg/kg, 3 days) or PCP plus clozapine (5 mg/kg, 3 days). (B) Representative current traces taken from the records used to construct panel A (at time points denoted by #). (C) Cumulative data (mean ± SEM showing the percentage reduction of NMDAR current amplitudes by PD168077 in PFC neurons from rats treated with PCP ($n = 7$), PCP plus clozapine ($n = 19$) or saline ($n = 12$). *$P < 0.01$, t test. (D) Plot of peak evoked NMDAR-EPSCs as a function of time and agonist (PD168077, 20 μM) application in two representative neurons from rats treated with PCP alone (5 mg/kg, 3 days) or PCP plus clozapine (5 mg/kg, 3 days). Each point represents the average peak (mean ± SEM) of 3 consecutive NMDAR-EPSCs. Scale bars: 50 pA, 200 ms. (F) Cumulative data (mean ± SEM) showing the percentage reduction of NMDAR-EPSC amplitudes by PD168077 in PFC neurons from rats treated with subchronic PCP ($n = 8$), PCP plus clozapine ($n = 11$) or saline ($n = 5$). *$P < 0.01$, t test.
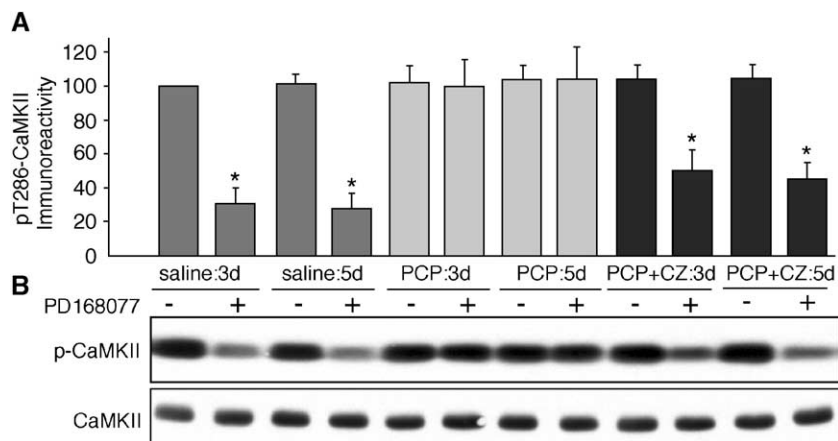

Fig. 7. The D_4 regulation of CaMKII activity was abolished in PCP-treated rats, and was restored after clozapine treatment. (A) Quantification of CaMKII Thr[286] phosphorylation in the absence or presence of PD168077 (40 μM) in PFC slices from rats treated with saline, PCP (5 mg/kg) or PCP plus clozapine (CZ, 5 mg/kg) for different durations. Each bar is the summary of 4–6 experiments. *$P < 0.01$, t test. (B) Representative immunoblots of Thr[286]-autophosphorylated CaMKII and total CaMKII in PFC slices from rats treated with saline, PCP or PCP plus clozapine.

that D_4-like receptors are up-regulated in PFC of schizophrenia patients (Seeman et al., 1993). However, reported changes on D_4 mRNA levels in PFC of schizophrenics have been conflicting (Roberts et al., 1996; Meador-Woodruff et al., 1997; Stefanis et al., 1998). Nevertheless, it is unclear whether actual alterations in functional D_4 receptors occur under pathological conditions. In this study, we demonstrate that the D_4 modulation of NMDA receptors in PFC is significantly impaired in rats after PCP exposure. The impairment is similar in rats treated acutely (single-dose) or subchronically (3–5 days) with PCP. The unaltered 5-HT$_{1A}$ and D_1 modulation of NMDA receptors in PCP-treated rats suggests that systemic administration of PCP does not produce a generally dysfunctional NMDA receptor that resists any regulation at the cellular level. Moreover, it suggests that not all receptors are impaired in the same way following systemic administration of PCP. Since the loss of D_4 functions is selective for NMDA receptors, but not for another target of D_4 receptors—voltage-dependent Ca^{2+} channels (VDCCs), it suggests that the expression of D_4 receptors is not significantly altered, but certain specific functions of D_4 receptors are disrupted after PCP exposure. The loss of D_4 receptor functions in PFC pyramidal neurons after PCP administration is consistent with the decreased functional activity of dopamine in the neocortex of schizophrenia (Davis et al., 1991; Akil et al., 1999), which is thought to mediate negative symptoms (Lewis and Lieberman, 2000).

The altered D_4 regulation of NMDAR functions after PCP administration is recovered by treatment with the antipsychotic drug clozapine. The ability of clozapine to alleviate negative symptoms of schizophrenia without inducing extrapyramidal side effects has been related to its higher affinity for D_4 receptors compared with D_2/D_3 receptors (Wilson et al., 1998). In addition to targeting D_4 receptors in PFC, clozapine also interacts with other receptors, such as serotonin receptors, muscarinic receptors and norepinephrine receptors (Oak et al., 2000), which may explain its better efficacy in treating schizophrenia than any agonists or antagonists selective to a single receptor subtype. It has been found that clozapine blocks behavioral impairments induced by NMDAR antagonists, but it lacks affinity for either ionotropic or metabotropic glutamate receptors (Lewis and Lieberman, 2000). Therefore, clozapine may be acting through dopamine receptors or other receptors to modify glutamate-mediated function and behavior. The results provided by this study suggest that the dysregulation of NMDA receptors could be one important mechanism for the dopamine dysfunction and the aberrant PFC activity in schizophrenia, and restoring the D_4 modulation of NMDAR channels in PFC could be one of the antipsychotic actions of clozapine.

A potential mechanism underlying the altered D_4 regulation of NMDAR transmission in PCP-treated rats is the disruption of signaling events linking D_4 receptors to NMDAR channels. Our previous study has found that the D_4 modulation of NMDAR functions in PFC is through a cascade involving the inhibition of PKA, activation of PP1 and ensuing inactivation of CaMKII, leading to the reduced surface expression of NMDA receptors (Wang et al., 2003). In this study, we demonstrated that the ability of D_4 receptors to inhibit CaMKII activity was significantly attenuated following PCP exposure. Given the intact D_4 modulation of VDCCs, which is mediated by a direct G protein action, it suggests that the D_4 coupling of G proteins is not impaired by PCP treatment. The second-messenger cascade downstream of G proteins appears to be disturbed in the PCP model of schizophrenia. One possible site of action for PCP is the

components of cAMP signaling. Clinical studies have found that adenylate cyclase and the PKA regulatory subunits were significantly reduced in schizophrenia patients compared with controls (Dean et al., 1997; Tardito et al., 2000), suggesting that schizophrenic patients have altered PKA levels. Another possible site is CaMKII. α-CaMKII knockout mice, especially the heterozygotes, exhibit a myriad of behavioral abnormalities including a decreased fear response and an increase in defensive aggression (Chen et al., 1994). These neuropsychiatric behavioral phenotypes exhibited in α-CaMKII$^{+/-}$ mice suggest that impaired CaMKII functions are involved in schizophrenia. Elevated CaMKII mRNA has been found in PFC from schizophrenia patients (Novak et al., 2000). However, little is known about how the properties and functions of CaMKII are dysregulated in neuropsychiatric disorders. Our current results provide the first evidence showing that the D_4 regulation of CaMKII is impaired after PCP exposure, and this dysregulation of CaMKII activity is restored by antipsychotic drug treatment.

Based on the experimental results, we propose a working model as follows. Dopamine input acts on D_4 receptors in PFC neurons to attenuate the activity of NMDA receptors, and this regulation is essential for the maintenance of a normal mental state. Administration of PCP interferes with D_4 receptor-mediated intracellular signaling pathways, thereby disrupting the D_4 modulation of NMDA receptors. The altered NMDAR functions may impair normal PFC neuronal activities required for cognition, reasoning, perception and emotion, leading to psychotic symptoms manifested in schizophrenia.

Experimental methods

Animals and drug administration

Male Sprague–Dawley young adult (3–4 weeks postnatal) rats were used as subjects. Phencyclidine hydrochloride (PCP, Sigma) was administered at a dose of 5 mg/kg in sterile saline daily. This dose of PCP administration to rats has been shown to induce a variety of behavioral and biochemical changes reminiscent of schizophrenia in humans (Jentsch et al., 1997b, 1998; Adams and Moghaddam, 1998; Moghaddam and Adams, 1998). Injections were given at a volume of 1 ml/kg intraperitoneally and vehicle treatments represented an injection of an equivalent volume of sterile saline. For acute PCP treatment, the rats were sacrificed 1 h or 1 day after i.p. injection of a single-dose of PCP. For subchronic PCP treatment, the rats were i.p. injected once daily with PCP for 3–5 days and sacrificed 24 h after the last injection. In a set of experiments, rats were i.p. injected once daily with clozapine at the standard prescription level (5 mg/kg, Malhotra et al., 1997) during the subchronic treatment with PCP.

Acute-dissociation procedure

PFC neurons were acutely dissociated using procedures similar to those described previously (Feng et al., 2001; Wang et al., 2003). All experiments were carried out with the approval of State University of New York at Buffalo Animal Care Committee. After incubation of brain slices in a NaHCO$_3$-buffered saline, PFC was dissected and placed in an oxygenated chamber containing papain (Sigma, 0.4 mg/ml) in HEPES-buffered Hank's balanced salt solution (HBSS, Sigma Chemical Co.) at 35°C. After 20–40 min

of enzyme digestion, tissue was rinsed three times in the low Ca^{2+}, HEPES-buffered saline and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was then plated into a 35 mm Lux Petri dish which was then placed on the stage of a Nikon inverted microscope.

Whole-cell recordings

Whole-cell recordings of currents employed standard voltage-clamp techniques (Wang et al., 2003; Chen et al., 2004). The internal solution consisted of (in mM): 180 N-methyl-D-glucamine (NMG), 40 HEPES, 4 $MgCl_2$, 0.1 BAPTA, 12 phosphocreatine, 2 Na_2ATP, 0.2 Na_3GTP, 0.1 leupeptin, pH 7.2–7.3, 265–270 mosM/l. The external solution consisted of (in mM): 127 NaCl, 20 CsCl, 10 HEPES, 1 $CaCl_2$, 5 $BaCl_2$, 12 glucose, 0.001 TTX, 0.02 glycine, pH 7.3–7.4, 300–305 mosM/l.

Recordings were obtained with an Axon Instruments 200B patch clamp amplifier that was controlled and monitored with an IBM PC running pCLAMP (v. 8) with a DigiData 1320 series interface (Axon instruments, Foster City, CA). Electrode resistances were typically 2–4 MΩ in the bath. After seal rupture, series resistance (4–10 MΩ) was compensated (70–90%) and periodically monitored. Care was exercised to monitor the constancy of the series resistance, and recordings were terminated whenever a significant increase (>20%) occurred. The cell membrane potential was held at −60 mV. The application of NMDA (100 μM) evoked a partially desensitizing inward current. Peak values were measured for generating the plot as a function of time and drug application. NMDA was applied for 2 s every 30 s to minimize desensitization-induced decrease of current amplitude. Drugs were applied with a gravity-fed 'sewer pipe' system. The array of application capillaries (ca. 150 μm i.d.) was positioned a few hundred microns from the cell under study. Solution changes were effected by the SF-77B fast-step solution stimulus delivery device (Warner Instrument Co., Hamden, CT).

Data analyses were performed with AxoGraph (Axon instruments, Union City, CA), Kaleidagraph (Albeck Software, Reading, PA), Origin 6 (OriginLab Co., Northampton, MA) and Statview (Abacus Concepts, Inc.). For analysis of statistical significance, Mann–Whitney U tests were performed to compare the current amplitudes in the presence or absence of agonists. Student's t tests were performed to compare the differential degrees of current modulation between groups subjected to PCP vs. saline treatment. In some cases, rats treated with PCP plus clozapine were compared with rats treated with PCP alone.

Electrophysiological recordings in slices

To evaluate the regulation of NMDAR-mediated excitatory postsynaptic currents by D_4 receptors in PFC slices, the whole-cell patch technique (Zhong et al., 2003; Wang et al., 2003) was used for voltage-clamp recordings using patch electrodes (5–9 MΩ) filled with the following internal solution (in mM): 130 Cs-methanesulfonate, 10 CsCl, 4 NaCl, 10 HEPES, 1 $MgCl_2$, 5 EGTA, 2.2 QX-314, 12 phosphocreatine, 5 MgATP, 0.2 Na_3GTP, 0.1 leupeptin, pH 7.2–7.3, 265–270 mosM/l. The slice (300 μm) was placed in a perfusion chamber attached to the fixed-stage of an upright microscope (Olympus) and submerged in continuously flowing oxygenated artificial cerebrospinal fluid (ACSF). Cells were visualized with a 40× water-immersion lens and illuminated with near infrared (IR) light and the image was detected with an IR-sensitive CCD camera. A Multiclamp 700A amplifier was used

for these recordings. Tight seals (2–10 GΩ) from visualized pyramidal neurons were obtained by applying negative pressure. The membrane was disrupted with additional suction and the whole cell configuration was obtained. The access resistances ranged from 13–18 MΩ. For recording NMDAR-mediated EPSCs, cells were bathed in ACSF containing CNQX (20 μM) and bicuculline (10 μM) continuously to block AMPA/kainate receptors and $GABA_A$ receptors. Evoked currents were generated with a 50 μs pulse from a stimulation isolation unit controlled by a S48 pulse generator (Astro-Med, Inc., West Warwick, RI). A bipolar stimulating electrode (FHC, Inc., Bowdoinham, ME) was positioned ~100 μm from the neuron under recording. Before stimulation, cells (voltage-clamped at −70 mV) were depolarized to +60 mV for 3 s to fully relieve the voltage-dependent Mg^{2+} block of NMDAR channels (Hestrin et al., 1990). Clampfit Program (Axon Instrument) was used to analyze evoked synaptic activity. The amplitude of EPSC was calculated by taking the mean of a 2–4 ms window around the peak and comparing with the mean of a 4–8 ms window immediately before the stimulation artifact.

Western blot analysis

PFC slices were prepared as previously described (Gu et al., 2003). Equal amounts of protein from slice homogenates were separated on 7.5% acrylamide gels and transferred to nitrocellulose membranes. The blots were blocked with 5% nonfat dry milk for 1 h at room temperature. Then the blots were incubated with the anti-Thr^{286}-phosphorylated CaMKII antibody (Promega, 1:2000) for 1 h at room temperature. After being rinsed, the blots were incubated with horseradish peroxidase-conjugated anti-rabbit antibodies (Amersham, 1:2000) for 1 h at room temperature. Following 3 washes, the blots were exposed to the enhanced chemiluminescence substrate. Then the blots were stripped for 1 h at 50°C followed by saturation in 5% nonfat dry milk and incubated with an anti-CaMKII antibody (Upstate Biotechnology, 1:2000) for the detection of the total CaMKII. Quantitation was obtained from densitometric measurements of immunoreactive bands on films. Levels of p-CaMKII or CaMKII with vs. without D_4 agonist in rats under different treatments were compared with Student's t tests.

References

Adams, B., Moghaddam, B., 1998. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. J. Neurosci. 18, 5545–5554.

Akil, M., Pierri, J.N., Whitehead, R.E., Edgar, C.L., Mohila, C., Sampson, A.R., Lewis, D.A., 1999. Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. Am. J. Psychiatry 156, 1580–1589.

Allen, R.M., Young, S.J., 1978. Phencyclidine-induced psychosis. Am. J. Psychiatry 135, 1081–1084.

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Andreasen, N.C., O'Leary, D.S., Flaum, M., Nopoulos, P., Watkins, G.L., Boles Ponto, L.L., Hichwa, R.D., 1997. Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. Lancet 349, 1730–1734.

Bakshi, V.P., Swerdlow, N.R., Geyer, M.A., 1994. Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. J. Pharmacol. Exp. Ther. 271, 787–794.

Carlsson, A., 1974. Antipsychotic drugs and catecholamine synapses. J. Psychiatr. Res. 11, 57–64.

Carlsson, A., 1988. The current status of the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1, 179–186.

Chen, C., Rainnie, D.G., Greene, R.W., Tonegawa, S., 1994. Abnormal fear response and aggressive behavior in mutant mice deficient for alpha-calcium-calmodulin kinase II. Science 266, 291–294.

Chen, G., Greengard, P., Yan, Z., 2004. Potentiation of NMDA receptor currents by dopamine D1 receptors in prefrontal cortex. Proc. Natl. Acad. Sci. U. S. A. 101, 2596–2600.

Cochran, S.M., Kennedy, M., McKerchar, C.E., Steward, L.J., Pratt, J.A., Morris, B.J., 2003. Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: differential modulation by antipsychotic drugs. Neuropsychopharmacology 28, 265–275.

Creese, I., Burt, D.R., Snyder, S.H., 1976. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192, 481–483.

Davis, K.L., Kahn, R.S., Ko, G., Davidson, M., 1991. Dopamine in schizophrenia: a review and reconceptualization. Am. J. Psychiatry 148, 1474–1486.

Dean, B., Opeskin, K., Pavey, G., Hill, C., Keks, N., 1997. Changes in protein kinase C and adenylate cyclase in the temporal lobe from subjects with schizophrenia. J. Neural Transm. 104, 1371–1381.

Deutch, A.Y., 1992. The regulation of subcortical dopamine systems by the prefrontal cortex: interactions of central dopamine systems and the pathogenesis of schizophrenia. J. Neural Transm., Suppl. 36, 61–89.

Deutch, A.Y., Tam, S.Y., Freeman, A.S., Bowers Jr., M.B., Roth, R.H., 1987. Mesolimbic and mesocortical dopamine activation induced by phencyclidine: contrasting pattern to striatal response. Eur. J. Pharmacol. 134, 257–264.

Dulawa, S.C., Grandy, D.K., Low, M.J., Paulus, M.P., Geyer, M.A., 1999. Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. J. Neurosci. 19, 9550–9556.

Feng, J., Cai, X., Zhao, J.H., Yan, Z., 2001. Serotonin receptors modulate GABAA receptor channels through activation of anchored protein kinase C in prefrontal cortical neurons. J. Neurosci. 21, 6502–6511.

Freeman, A.S., Bunney, B.S., 1984. The effects of phencyclidine and N-allylnormetazocine on midbrain dopamine neuronal activity. Eur. J. Pharmacol. 104, 287–293.

Glase, S.A., Akunne, H.C., Georgic, L.M., Heffner, T.G., MacKenzie, R.G., Manley, P.J., Pugsley, T.A., Wise, L.D., 1997. Substituted [(4-phenylpiperazinyl)-methyl]benzamides: selective dopamine D4 agonists. J. Med. Chem. 40, 1771–1772.

Goldman-Rakic, P.S., 1994. Working memory dysfunction in schizophrenia. J. Neuropsychiatry Clin. Neurosci. 6, 348–357.

Grace, A.A., 1991. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41, 1–24.

Gu, Z., Zhong, P., Yan, Z., 2003. Activation of muscarinic receptors inhibits β-amyloid peptide-induced signaling in cortical slices. J. Biol. Chem. 278, 17546–17556.

Handelmann, G.E., Contreras, P.C., O'Donohue, T.L., 1987. Selective memory impairment by phencyclidine in rats. Eur. J. Pharmacol. 140, 69–73.

Hestrin, S., Nicoll, R.A., Perkel, D.J., Sah, P., 1990. Analysis of excitatory synaptic action in pyramidal cells using whole-cell recording from rat hippocampal slices. J. Physiol. 422, 203–225.

Hille, B., 1994. Modulation of ion-channel function by G-protein-coupled receptors. Trends Neurosci. 17, 531–536.

Hondo, H., Yonezawa, Y., Nakahara, T., Nakamura, K., Hirano, M., Uchimura, H., Tashiro, N., 1994. Effect of phencyclidine on dopamine release in the rat prefrontal cortex; an in vivo microdialysis study. Brain Res. 633, 337–342.

Itil, T., Keskiner, A., Kiremitci, N., Holden, J.M.C., 1967. Effect of phencyclidine in chronic schizophrenics. Can. J. Psychiatry 12, 209–212.

Javitt, D.C., Zukin, S.R., 1991. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry 148, 1301–1308.

Jentsch, J.D., Roth, R.H., 1999. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20, 201–225.

Jentsch, J.D., Elsworth, J.D., Redmond Jr., D.E., Roth, R.H., 1997a. Phencyclidine increases forebrain monoamine metabolism in rats and monkeys: modulation by the isomers of HA966. J. Neurosci. 17, 1769–1775.

Jentsch, J.D., Tran, A., Le, D., Youngren, K.D., Roth, R.H., 1997b. Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. Neuropsychopharmacology 17, 92–99.

Jentsch, J.D., Tran, A., Taylor, J.R., Roth, R.H., 1998. Prefrontal cortical involvement in phencyclidine-induced activation of the mesolimbic dopamine system: behavioral and neurochemical evidence. Psychopharmacology (Berlin) 138, 89–95.

Krystal, J.H., Karper, L.P., Seibyl, J.P., Freeman, G.K., Delaney, R., Bremner, J.D., Heninger, G.R., Bowers Jr., M.B., Charney, D.S., 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry 51, 199–214.

Lahti, A.C., Koffel, B., LaPorte, D., Tamminga, C.A., 1995. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13, 9–19.

Lewis, D.A., Lieberman, J.A., 2000. Catching up on schizophrenia: natural history and neurobiology. Neuron 28, 325–334.

Luby, E.D., Cohen, B.D., Rosenbaum, G., Gottlieb, J.S., Kelly, R., 1959. Study of a new schizophrenic-like drug: sernyl. Arch. Neurol. Psychiatry 81, 363–369.

Malhotra, A.K., Adler, C.M., Kennison, S.D., Elman, I., Pickar, D., Breier, A., 1997. Clozapine blunts N-methyl-D-aspartate antagonist-induced psychosis: a study with ketamine. Biol. Psychiatry 42, 664–668.

Meador-Woodruff, J.H., Haroutunian, V., Powchik, P., Davidson, M., Davis, K.L., Watson, S.J., 1997. Dopamine receptor transcript expression in striatum and prefrontal and occipital cortex. Focal abnormalities in orbitofrontal cortex in schizophrenia. Arch. Gen. Psychiatry 54, 1089–1095.

Miller, S.G., Kennedy, M.B., 1986. Regulation of brain type II Ca2+/calmodulin-dependent protein kinase by autophosphorylation: a Ca2+-triggered molecular switch. Cell 44, 861–870.

Moghaddam, B., Adams, B.W., 1998. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. Science 281, 1349–1352.

Moghaddam, B., Adams, B., Verma, A., Daly, D., 1997. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J. Neurosci. 17, 2921–2927.

Mohn, A.R., Gainetdinov, R.R., Caron, M.G., Koller, B.H., 1999. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell 98, 427–436.

Mrzljak, L., Bergson, C., Pappy, M., Huff, R., Levenson, R., Goldman-Rakic, P.S., 1996. Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. Nature 381, 245–248.

Novak, G., Seeman, P., Tallerico, T., 2000. Schizophrenia: elevated mRNA for calcium-calmodulin-dependent protein kinase IIbeta in frontal cortex. Brain Res. Mol. Brain Res. 82, 95–100.

Oak, J.N., Oldenhof, J., Van Tol, H.H., 2000. The dopamine D(4) receptor: one decade of research. Eur. J. Pharmacol. 405, 303–327.

Olney, J.W., Newcomer, J.W., Farber, N.B., 1999. NMDA receptor hypofunction model of schizophrenia. J. Psychiatr. Res. 33, 523–533.

Raja, S.N., Guyenet, P.G., 1980. Effects of phencyclidine on the spontaneous activity of monoaminergic neurons. Eur. J. Pharmacol. 63, 229–233.

Roberts, D.A., Balderson, D., Pickering-Brown, S.M., Deakin, J.F., Owen, F., 1996. The relative abundance of dopamine D4 receptor mRNA in post mortem brains of schizophrenics and controls. Schizophr. Res. 20, 171–174.

Rubinstein, M., Phillips, T.J., Bunzow, J.R., Falzone, T.L., Dziewczapolski, G., Zhang, G., Fang, Y., Larson, J.L., McDougall, J.A., Chester, J.A., Saez, C., Pugsley, T.A., Gershanik, O., Low, M.J., Grandy, D.K., 1997. Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell 90, 991–1001.

Rubinstein, M., Cepeda, C., Hurst, R.S., Flores-Hernandez, J., Ariano, M.A., Falzone, T.L., Kozell, L.B., Meshul, C.K., Bunzow, J.R., Low, M.J., Levine, M.S., Grandy, D.K., 2001. Dopamine D4 receptor-deficient mice display cortical hyperexcitability. J. Neurosci. 21, 3756–3763.

Sawa, A., Snyder, S.H., 2002. Schizophrenia: diverse approaches to a complex disease. Science 296, 692–695.

Seeman, P., Lee, T., Chau-Wong, M., Wong, K., 1976. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261, 717–719.

Seeman, P., Guan, H.C., Van Tol, H.H., 1993. Dopamine D4 receptors elevated in schizophrenia. Nature 365, 441–445.

Snyder, S.H., 1980. Phencyclidine. Nature 285, 355–356.

Stefanis, N.C., Bresnick, J.N., Kerwin, R.W., Schofield, W.N., McAllister, G., 1998. Elevation of D4 dopamine receptor mRNA in postmortem schizophrenic brain. Brain Res. Mol. Brain Res. 53, 112–119.

Steinpreis, R.E., Sokolowski, J.D., Papanikolaou, A., Salamone, J.D., 1994. The effects of haloperidol and clozapine on PCP- and amphetamine-induced suppression of social behavior in the rat. Pharmacol. Biochem. Behav. 47, 579–585.

Tamminga, C.A., 1998. Schizophrenia and glutamatergic transmission. Crit. Rev. Neurobiol. 12, 21–36.

Tardito, D., Tura, G.B., Bocchio, L., Bignotti, S., Pioli, R., Racagni, G., Perez, J., 2000. Abnormal levels of cAMP-dependent protein kinase regulatory subunits in platelets from schizophrenic patients. Neuropsychopharmacology 23, 216–219.

Van Tol, H.H., Bunzow, J.R., Guan, H.C., Sunahara, R.K., Seeman, P., Niznik, H.B., Civelli, O., 1991. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 350, 610–614.

Verma, A., Moghaddam, B., 1996. NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine. J. Neurosci. 16, 373–379.

Wang, X., Zhong, P., Yan, Z., 2002. Dopamine D4 receptors modulate GABAergic signaling in pyramidal neurons of prefrontal cortex. J. Neurosci. 22, 9185–9193.

Wang, X., Zhong, P., Gu, Z., Yan, Z., 2003. Regulation of NMDA receptors by dopamine D4 signaling in prefrontal cortex. J. Neurosci. 23, 9852–9861.

Wedzony, K., Chocyk, A., Mackowiak, M., Fijal, K., Czyrak, A., 2000. Cortical localization of dopamine D4 receptors in the rat brain-immunocytochemical study. J. Physiol. Pharmacol. 51, 205–221.

Weinberger, D., Berman, K., Zec, R., 1986. Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia: I. Regional cerebral blood flow evidence. Arch. Gen. Psychiatry 43, 114–124.

Wilson, J.M., Sanyal, S., Van Tol, H.H.M., 1998. Dopamine D-2 and D-4 receptor ligands: relation to antipsychotic action. Eur. J. Pharmacol. 351, 273–286.

Yan, Z., Song, W.J., Surmeier, D.J., 1997. D2 dopamine receptors reduce N-type Ca^{2+} currents in rat neostriatal cholinergic interneurons through a membrane-delimited, protein kinase C-insensitive pathway. J. Neurophysiol. 77, 1003–1015.

Yuen, E.Y., Jiang, Q., Chen, P., Gu, Z., Feng, J., Yan, Z., 2005. Serotonin 5-HT1A receptors regulate NMDA receptor channels through a microtubule-dependent mechanism. J. Neurosci. 25, 5488–5501.

Zhong, P., Gu, Z., Wang, X., Jiang, H., Feng, J., Yan, Z., 2003. Impaired modulation of GABAergic transmission by muscarinic receptors in a mouse transgenic model of Alzheimer's disease. J. Biol. Chem. 278, 26888–26896.