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Abstract

In situ hybridization of slide-mounted brain sections from rats subjected to acute and chronic phencyclidine treatment was carried out using synthetic oligonucleotides complementary to dopamine D2-receptor and non-N-methyl-D-aspartate (NMDA) glutamate-receptor-subunit (GluR-1) mRNAs. There was no significant difference in either the D2-receptor or the GluR-1 mRNA levels in any brain region of the acute phencyclidine (10 mg/kg)-treated and control groups. However, chronic administration of phencyclidine (10 mg/kg/day, 14 days) significantly decreased the dopamine D2-receptor mRNA level in the caudate-putamen (by 27%, $P < 0.01$) and significantly increased the GluR-1 mRNA level in the prefrontal cortex (by 29%, $P < 0.001$). These results suggest that the chronic pharmaco-behavioral effects of phencyclidine may involve expression of both dopamine- and non-NMDA glutamate-receptor mRNAs.

KEYWORDS: dopamone D2 receptor, GluR-1 glutamate reseptor, mRNA, phencyclidine, in situ hybridization

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Changes in Dopamine D_2 and GluR-1 Glutamate Receptor mRNAs in the Rat Brain after Treatment with Phencyclidine

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In situ hybridization of slide-mounted brain sections from rats subjected to acute and chronic phencyclidine treatment was carried out using synthetic oligonucleotides complementary to dopamine D_2-receptor and non-N-methyl-D-aspartate (NMDA) glutamate-receptor-subunit (GluR-1) mRNAs. There was no significant difference in either the D_2-receptor or the GluR-1 mRNA levels in any brain region of the acute phencyclidine (10mg/kg)-treated and control groups. However, chronic administration of phencyclidine (10mg/kg/day, 14 days) significantly decreased the dopamine D_2-receptor mRNA level in the caudate-putamen (by 27%, $P < 0.01$) and significantly increased the GluR-1 mRNA level in the prefrontal cortex (by 29%, $P < 0.001$). These results suggest that the chronic pharmaco-behavioral effects of phencyclidine may involve expression of both dopamine- and non-NMDA glutamate-receptor mRNAs.

Key words: dopamine D_2 receptor, GluR-1 glutamate receptor, mRNA, phencyclidine, *in situ* hybridization

Phencyclidine [1-(1-phenylcyclohexyl)piperidine; PCP], a general anesthetic, was first synthesized in 1957 (1) and has been reported to induce dissociation from the environment without complete loss of consciousness (2). Therefore, PCP and its derivatives were called "dissociative anesthetics". Psychotic reactions, including agitation, excitement, bizarre behavior, paranoia and hallucinations, were observed in patients given PCP. Not only does PCP induce agitation, paranoia and hallucinations, which resemble positive schizophrenic symptoms, but it also causes looseness of association, loss of ego boundaries and concreteness, which resemble the core symptoms of schizophrenia (2).

The mechanisms by which PCP exerts such psychological effects have yet to be elucidated. In a receptor binding study, it was demonstrated that PCP binding sites were located within the cation channels of the N-methyl-D-aspartate (NMDA) receptor complex (3). The NMDA receptor interacts with excitatory amino acids in the brain and PCP inhibits glutamatergic neurotransmission by blocking the NMDA receptor ion-channel complex noncompetitively (3). Therefore, research into the mechanisms of PCP-induced psychosis has led to a hypotheses that endogenous dysfunction of NMDA receptor-mediated neurotransmission might contribute to the pathogenesis of schizophrenia (2). However there are few reports about the effects of PCP on the non-NMDA receptor-mediated neurotransmission.

Many workers have suggested that there is an interaction between the PCP-binding sites and the dopaminergic system. For example, MK-801, a noncompetitive NMDA-receptor antagonist, was demonstrated to increase dopamine metabolism in the rat brain (4). Therefore, PCP-induced psychosis has led to a hypothesis which has resulted in the development of experimental models for neurochemical investigations of the underlying dopaminergic and glutamatergic dysfunction in schizophrenia (2).

In this study, to reveal the effect of PCP on dopaminergic and non-NMDA receptor-mediated neurotransmission in aspect of the expression of receptor mRNA, *in situ* hybridization of rat brain sections was carried out using synthetic oligonucleotide probes complementary to dopamine D_2-receptor and non-NMDA glutamate-receptor-subunit (GluR-1) mRNAs after acute and chronic PCP treatment.

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Materials and Methods

Male Sprague-Dawley rats (Charles River, Japan), which weighed 240–260 g at the start of each experiment were used.

Experiment 1. To investigate the effects of acute administration of PCP, the experimental animals (N = 8) were injected with PCP (10 mg/kg i.p., diluted to 10 mg/ml with physiological saline) and killed by decapitation 60 min later. The controls (N = 8) were injected with physiological saline (1 ml/kg i.p.) and were killed in the same manner.

Experiment 2. To investigate the effects of chronic PCP treatment, the experimental and control animals (8 rats/group) were treated with PCP (10 mg/kg, diluted to 10 mg/ml with physiological saline) and physiological saline (1 ml/kg), respectively, every day for 14 days and were killed 60 min after the last injection.

In situ hybridization. The brains were removed and frozen immediately in powdered dry ice. Cryostat sections (10 μm) were mounted on gelatine-coated slides and stored at −20℃ until required for use. After the sections were fixed with phosphate-buffered saline (PBS), which contained 4 % (w/v) paraformaldehyde, for 5 min, rinsed twice for 3 min each with PBS, acetylated with 0.1 M triethanolamine in 0.9 % (w/v) NaCl (pH 8.0) containing 0.25 % (v/v) acetic anhydride, delipidated in a graded series of ethanol rinses, followed by incubation with chloroform for 5 min and finally air-dried. The hybridization buffer used was comprised of 50 % (w/v) formamide, 4 × SSC (1 × SSC contained 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 1 × Denhardt's solution, 10 % (w/v) dextran sulfate, 0.1 M dithiothreitol and 0.48 mg/ml sheared salmon sperm DNA. A 48-base synthetic oligonucleotide complementary to rat dopamine $D_{2(444)}$-receptor mRNA (5), which encodes bases 724 to 771 of the portion between the fifth and sixth transmembrane domains of the receptor polypeptide, was used as a probe to detect dopamine D_2-receptor mRNA. A 45-base synthetic oligonucleotide complementary to rat non-NMDA glutamate-receptor subunit (GluR-1) mRNA (6), which encodes bases 1642 to 1686 of the portion between the first and second transmembrane domains of the receptor polypeptide, was used as a probe to detect glutamate-receptor mRNA. "Randomer" (New England Nuclear), a probe which represents a random arrangement of 48 bases, was used to determine the non-specific accumulation of the oligonucleotide probes. The oligonucleotides were 3'-end labeled with α [^{35}S] d-adenosine triphosphate (ATP, specific activity 1374 Ci/mmol, New England Nuclear) using terminal deoxyribonucleotidyl transferase (3'-end labeling system, New England Nuclear) and purified on a Nensorb column (New England Nuclear), after which they were diluted with the hybridization buffer to a concentration of 1×10^6 dpm/100 μl and subjected to *in situ* hybridization (total volume: 100 μl/section) at 37 ℃ for 16 h in a humid chamber. After hybridization, the sections were rinsed 4 times for 15 min each with 1 × SSC at 37℃, 4 times for 15 min each with 1 × SSC at 20℃ and air-dried. Tritium-sensitive films (LKB) were exposed to the sections for 21 days and then developed. The same film was exposed to all the sections hybridized with the same probe in each experiment. The optical densities of the dopamine D_2 and GluR-1 receptor mRNAs on the autoradiographs were analyzed using a densitometer (Micro Image). Outlining was guided by reference to the corresponding Nissl-stained section and a rat brain atlas (7). The statistical significance of differences between the mean ± SD values of the experimental and control groups was evaluated using Student's *t*-test. Differences at P values of less than 0.05 were considered to be significant.

Results

Experiment 1. Representative autoradiographs of dopamine D_2-receptor mRNA in the rat brain are shown in Fig. 1. High levels of this mRNA were observed in the caudate-putamen and substantia nigra pars compacta. Slight accumulation of the "Randomer" probe in the hippocampal formation was observed, but otherwise, its density throughout the whole brain was virtually identical (Fig. 2). The mean optical densities of dopamine D_2-receptor mRNA in each brain region of the acutely PCP-treated and control rats are shown in Fig. 3. The mean density of the dopamine D_2-receptor mRNA in every brain region of the acutely PCP-treated rats examined did not differ significantly from that in the corresponding region in the controls.

Representative autoradiographs of the GluR-1 mRNAs in the brains of rats subjected to acute treatment with PCP and saline are shown in Fig. 4. The highest levels of GluR-1 mRNA were observed in the hippocampal formation and moderate levels were present in the prefrontal and cingulate cortices. The mean optical

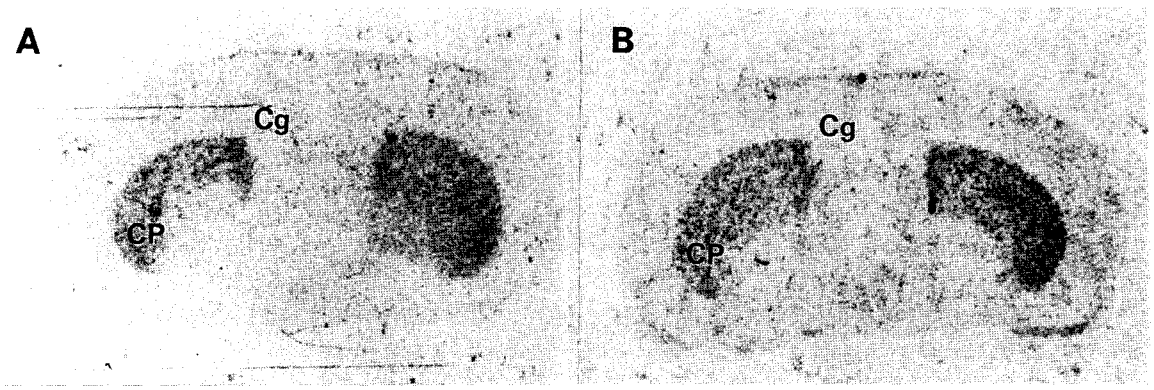

Fig. 1

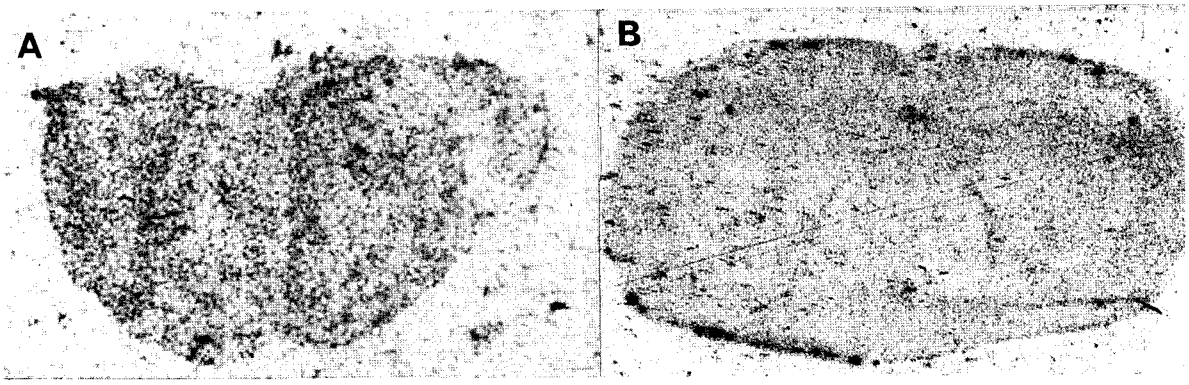

Fig. 2

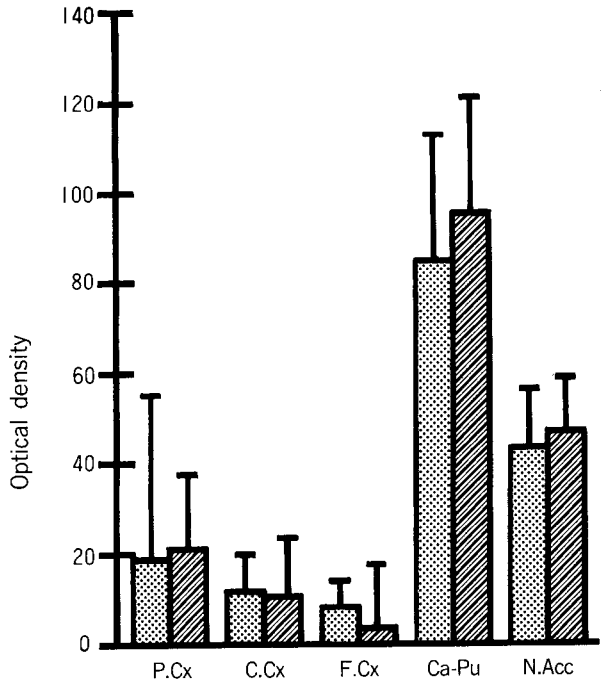

Fig. 3

Fig. 1 Representative autoradiographs of dopamine D_2-receptor mRNA in the brains of rats subjected to acute treatment with phencyclidine (PCP) and saline (controls). **A.** and **B.**: Sections at bregma −0.3 mm (× 6): **A.**: Control group; **B.**: PCP-treated group; Cg: Cingulate cortex; CP: Caudate-putamen.

Fig. 2 Representative autoradiographs of "Randomer" probe in the rat brains. **A.**: Section at bregma + 5.2 mm (× 10); **B.**: Section at bregma −0.3 mm (× 7).

Fig. 3 Relative amounts of dopamine D_2-receptor mRNA (mean optical densities ± SD) in each brain region following acute administration of PCP and saline. Each bar represents the result of *in situ* hybridization followed by film autoradiography and densitometric analysis. ⊡: Saline; ▨: PCP. P. Cx: Prefrontal cortex; C. Cx: Cingulate cortex; F. Cx: Frontal cortex; Ca-Pu: Caudate-Putamen; N. Acc: Nucleus accubens. PCP; See Fig. I.

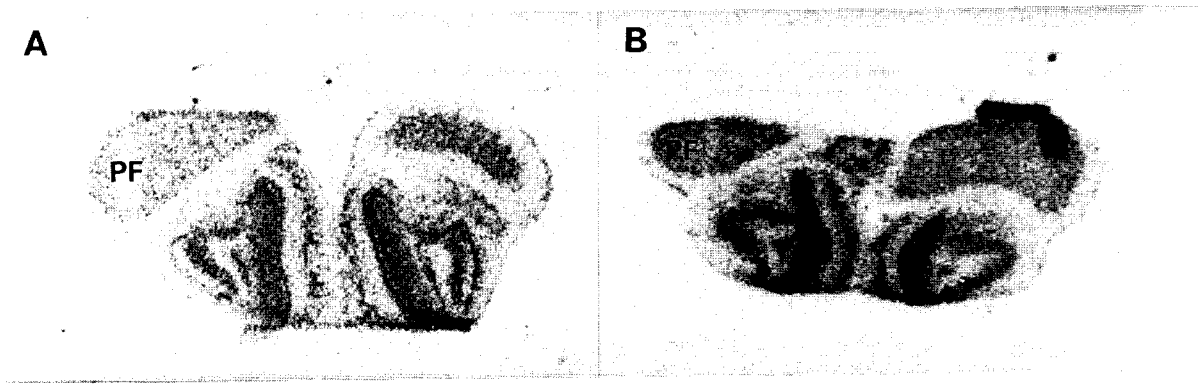

Fig. 4 Representative autoraiiographs of GluR-I mRNA in the brains of rats subjected to acute treament with PCP and saline (controls). **A.** and **B.**: Sections at bregma + 5.2 mm (× 9); **A.**: Control group; **B.**: PCP-treated group; PF: Prefrontal cortex. PCP: See Fig. 1.

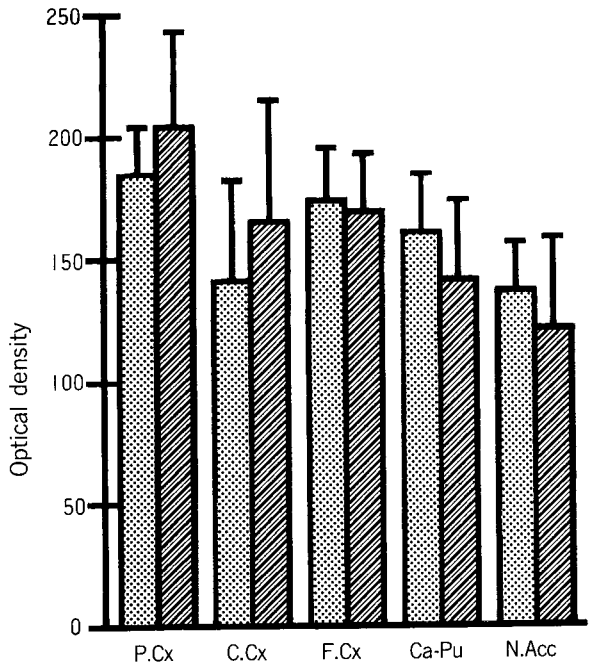

Fig. 5 Relative amounts of GluR-I mRNA (mean optical densities ± SD) in each brain region following acute administration of PCP and saline. Each bar represents the result of *in situ* hybridization followed by film autoradiography and densitometric analysis. Abbreviations: See Figs. 1, 3. ▨: Saline; ▨: PCP.

densities of GluR-1 mRNA in each brain region of the acutely PCP-treated and control rats are shown in Fig. 5. The mean optical density of GluR-1 mRNA in every brain region of the acutely PCP-treated rats examined did not differ significantly from that in the corresponding regions in the controls.

Experiment 2. In the study of the effect of chronic PCP treatment, the experimental and control animals were decapitated 60 min after the last injection of PCP or saline. The autoradiographs of dopamine D_2-receptor and GluR-1 mRNAs were similar to those obtained in Experiment 1 and representative examples are shown in Figs. 6 and 7, respectively. The mean optical densities of dopamine D_2-receptor and GluR-1 mRNAs in each brain region of the PCP-treated and control rats examined are shown in Figs. 8 and 9, respectively. The mean dopamine D_2-receptor mRNA density in the caudate-putamen of the PCP-treated rats 60 min after the last injection was significantly lower (by 27 %, $P < 0.01$) than that in the control rats (Fig. 8). The mean density of the GluR-1 mRNA in the prefrontal cortex of the PCP-treated rats 60 min after the last injection was significantly higher (by 29 %, $P < 0.001$) than that in the control rats. In the cingulate cortex, the mean GluR-1 mRNA density in the PCP-treated rats appeared to be higher than that in

Fig. 6 Representative autoradiographs of dopamine D_2-receptor mRNA in the brains of rats subjected to chronic treatment with phencyclidine (PCP) and saline (controls). **A.** and **B.**: Sections at bregma + 5.2 mm (× 9); **C.** and **D.**: Bregma − 0.3 mm (× 6); **A.** and **C.**: Control group; **B.** and **D.**: PCP-treated group; PF: Prefrontal cortex; Cg: Cingulate cortex; CP: Caudate-putamen.

Fig. 7 Representative autoradiographs of GluR-I mRNA in the brains of rats subjected to chronic treatment with PCP and saline (controls). **A.** and **B.**: sections at bregma + 5.2 mm (× 9); **C.** and **D.**: bregma − 0.3 mm (× 6), **A.** and **C.**: Control group; **B.** and **D.**: PCP-treated group; PF: Prefrontal cortex; Cg: Cingulate cortex; CP: Caudate-putamen. PCP: See Fig. 6.

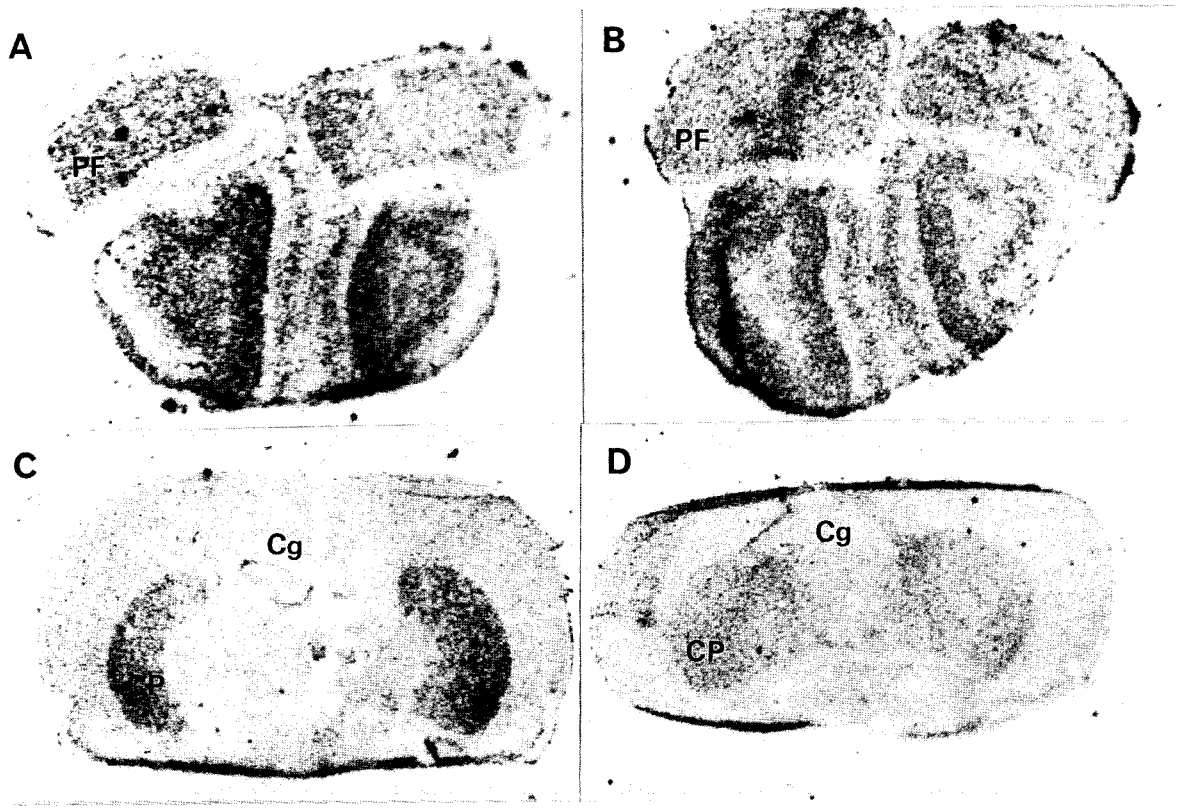

Fig. 6

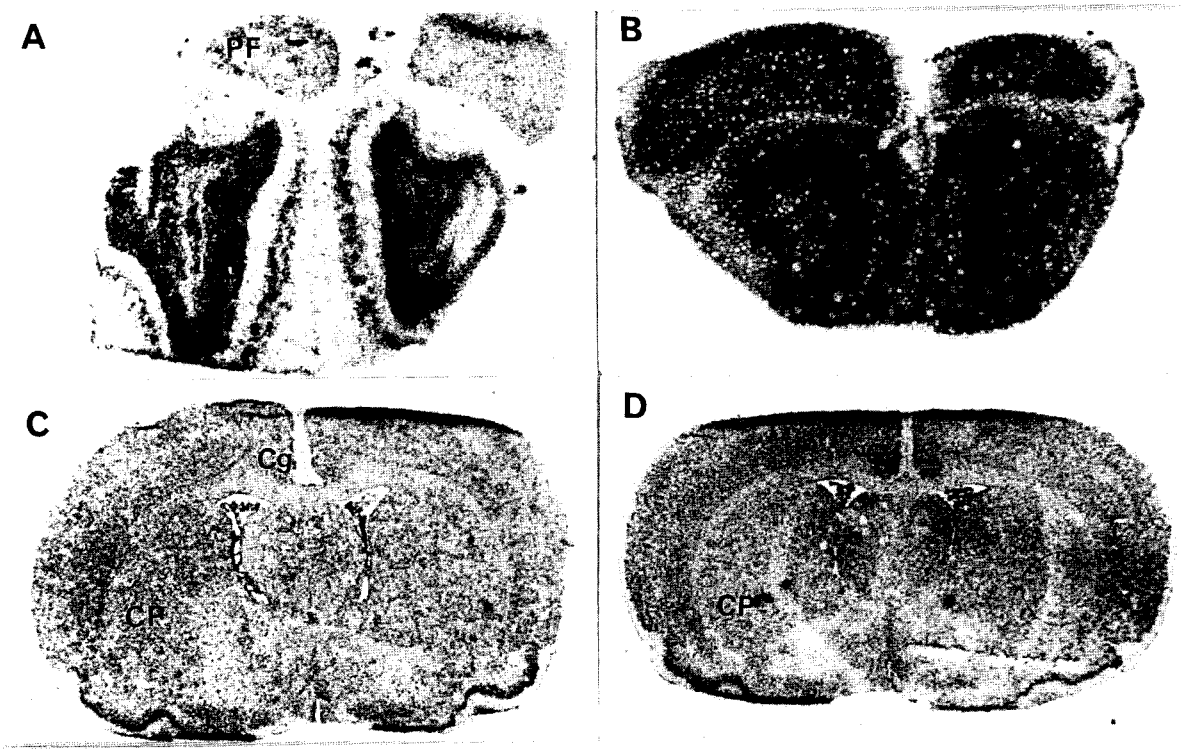

Fig. 7

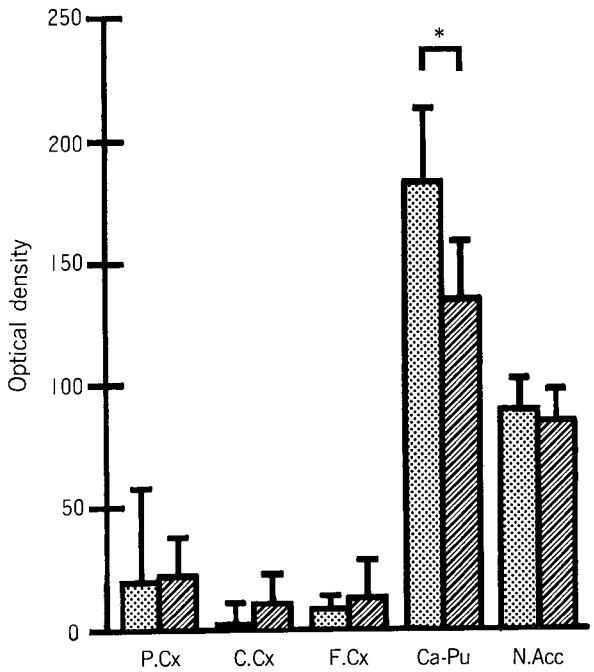

Fig. 8 Relative amounts of dopamine D_2-receptor mRNA (mean optical densities ± SD) in each brain region following chronic treatment with PCP and saline. Each bar represents the result of *in situ* hybridization followed by film autoradiography and densitometric analysis. Significant differences between the saline and PCP-treated groups are indicated by an asterisk ($* P < 0.01$). Abbreviations; See Figs. 1, 3. ▨: Saline; ▨: PCP.

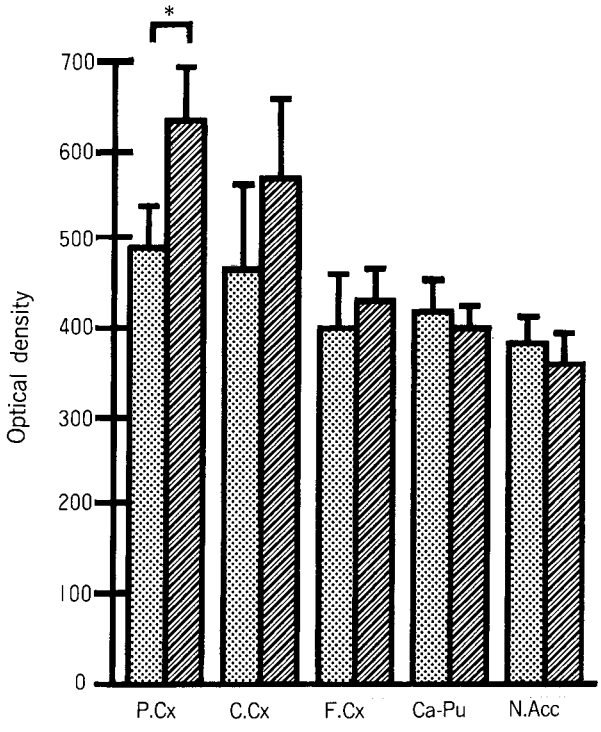

Fig. 9 Relative amounts of GluR-1 mRNA (mean optical densities ± SD) in each brain region following chronic treatment with PCP and saline. Each bar represents the result of *in situ* hybridization followed by film autoradiography and densitometric analysis. Significant differences between the saline and PCP-treated groups are indicated by an asterisk ($* P < 0.001$). Abbreviations: See Figs. 1, 3. ▨: Saline; ▨: PCP.

the controls, but failed to reach statistical significance ($P < 0.1$) (Fig. 9). There were no differences between the two groups with respect to the dopamine D_2-receptor and GluR-1 mRNA densities in any other brain region examined.

Discussion

The effects of chronic PCP administration on dopamine D_2 receptor binding have been reported (8, 9). The B_{max} of [^{3}H] spiperone (dopamine D_2 receptor antagonist) binding to rat striatal membranes decreased 24 h after the final injection of a chronic (28 days) PCP treatment regimen, although its affinity for the receptors (K_d) was similar to the control values (9). However, a subsequent study demonstrated that the B_{max} of [^{3}H] spiperone binding to dopamine D_2 receptors in the striata of rats after receiving PCP by continuous infusion for 7 days with a subsequent 2-day washout period did not differ significantly from those of saline-infused controls. The receptor density also was not significantly altered 45 min after acute administration of PCP (8). In our study, chronic PCP treatment inhibited the expression of dopamine-receptor mRNA, which supports the hypothesis that down-regulation of dopamine D_2 receptors induced by chronic PCP treatment occurs as a result of inhibition of mRNA expression.

The interactions of PCP with the mesocortical and mesostriatal dopaminergic systems have been the subject of a great deal of debate. Rao *et al.* reported that PCP increased dopamine release in the amygdala, pyriform and prefrontal cortex, but not in the striatum (10), whereas Carboni *et al.* reported that PCP increased dopamine release in the nucleus accumbens and striatum (11).

Furthermore, recent study demonstrated that PCP induced dopamine metabolism alterations in various parts of the brain, not just in the striatum (4). Therefore, a decrease in D_2-receptor mRNA expression may reflect an increase in dopamine release.

PCP is known to bind specifically to NMDA-sensitive receptor-linked cation channels and to inhibit glutamatergic neurotransmission by blocking the influx of calcium ions. However, there are few reports about the effects of PCP on the release of glutamate and non-NMDA glutamatergic neurotransmission. If the blockade of NMDA receptors achieved by PCP administration regulates glutamate release, then glutamate receptor binding may be altered by PCP administration.

In this study, an *in situ* hybridization technique using a synthetic oligonucleotide probe complementary to the mRNA of GluR-1, a glutamate receptor subunit, was employed to investigate whether glutamate receptor expression in the rat brain changed after PCP treatment. The GluR-1 subunit was the first of the glutamate receptor subunits to be cloned by Hollman in 1989 (5). Subsequently, a series of non-NMDA glutamate receptor subunits were cloned (12), the last of which was called KA-1 (kainic acid receptor-1) and the others GluR-1 through GluR-6. Further characterization revealed that GluR-1, -2, -3 and -4 are subunits of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, and that GluR-5 and 6 and KA-1 are involved with the kainate receptor (12).

A few reports about glutamate receptor binding after PCP treatment have been published. Kainate binding in the prefrontal cortex of the rat brain was reported to be increased by chronic administration of PCP (13). There are, to our knowledge, no reports about the expression of NMDA glutamate receptors, although there is one about [^{3}H] N-[1-(2-thienyl) cyclohexyl] piperidine (TCP) binding to NMDA-sensitive receptor-linked cation channels: the B_{max} of [^{3}H] TCP binding in the whole brain minus cerebellum increased significantly after PCP administration (14). The GluR-1 subunit detected in our study is believed to be involved with the AMPA, rather than the kainate, receptor (15). These results suggest that PCP administration may decrease glutamatergic transmission by both NMDA and non-NMDA glutamate receptors. Recently, systemically administered PCP was found to reduce K^+-evoked glutamate release in the rat anterior cingulate cortex significantly (16), which suggests that PCP may inhibit glutamate release through an NMDA receptor-mediated mechanism and, secondarily, facilitate the expression of non-NMDA-receptor mRNA.

In conclusion, chronic administration of PCP decreased dopamine D_2-receptor mRNA expression in the caudate-putamen of the rat brain and increased GluR-1 mRNA expression in the prefrontal cortex. These results suggest that the psychotomimetic effects induced by chronic PCP administration may be due to changes in the expression of dopamine- and non-NMDA glutamate-receptor mRNAs.

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