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Effects of nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester on phencyclidine-induced effects in rats

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Abstract

Phencyclidine (PCP) is widely used as an animal model of schizophrenia. In rats, acute PCP treatment increased locomotor activity and induced stereotyped behaviours consisting of head weaving, turning and backpedalling. PCP had differential regional effects on c-*fos* expression in rat brain, suggesting different patterns of neuronal activity. The most prominent immunostaining was observed in the cortical regions. To elucidate the role of nitric oxide, an important intracellular messenger, in the mechanism of action of PCP the effects of nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) were studied in PCP-treated animals. L-NAME potentiated PCP-induced behaviours and c-*fos* expression in many brain regions. The greatest increases were observed in the frontal, retrosplenial granular cortex, cerebellum, thalamic and subthalamic nuclei. While PCP alone induced low c-*fos* expression in the entorhinal cortex, with almost no expression in the rostral part of caudate putamen, animals pretreated with L-NAME showed marked activation in these brain areas. These results strongly indicate the involvement of the nitric oxide system in the mechanism of action of PCP.

Keywords: Phencyclidine; Nitric oxide (NO); N^G-nitro-L-arginine methyl ester; Behaviour; c-*fos*; (Rat)

1. Introduction

Several lines of evidence suggest that glutamatergic mechanisms play an important role in the pathophysiology of schizophrenia (Toru et al., 1994; Hirsch et al., 1997; Tamminga, 1998; Heresco-Levy and Javitt, 1998; Carlsson et al., 1999; Duncan et al., 1999). Included in this evidence is the fact that phencyclidine (PCP) and other antagonists of NMDA receptors such as ketamine and (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptane-5,10-imine maleate (MK-801) have psychotomimetic effects in normal individuals (Krystal et al., 1994; Duncan et al., 1999) and exacerbate pre-existing symptoms in schizophrenic patients (Lahti et al., 1995; Steinpreis, 1996; Duncan et al., 1999). Since PCP can mimic the full spectrum of schizophrenic disorders (Toru et al., 1994; Steinpreis, 1996; Thornberg and Saklad, 1996; Duncan et al., 1999), it has been proposed to be one of the best drug-induced models of schizophrenia. The preclinical pharmacology of PCP has been studied in animals for many years to determine potential neurochemical correlates of psychosis and to find new antipsychotic drugs (Sturgeon et al., 1979; Yang et al., 1991; Corbett et al., 1995; Noda et al., 1995b, 1996; Gleason and Shannon, 1997; Moghaddam and Adams, 1998; Johansson et al., 1999).

Nitric oxide (NO) is an important intracellular messenger in the central nervous system and may also operate as a neurotransmitter. Release of NO occurs as a consequence of glutamate stimulation of NMDA receptors and is dependent upon calcium-calmodulin activation of the enzyme NO synthase (NOS), (Ignarro and Murad, 1995; Szabo, 1996). NO mediates a range of different physiological functions. It has been implicated in various neuropathological conditions including schizophrenia (Akbarian et al., 1993; Karatinos et al., 1995; Khan et al., 1995; Das et al., 1995; Karson et al., 1996). The role of NO in these central processes has been elucidated primarily through the use of NOS inhibitors, of which N^G-nitro-L-arginine (L-NOARG)

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and its methyl ester (L-NAME) have been used most frequently. There are several reports indicating that NO may be involved in the mechanism of action of PCP, but its actual role is still unclear. Since NO may serve as an intracellular messenger for NMDA glutamatergic neurones, it is possible that NOS inhibitors may have synergistic effects with NMDA receptor antagonists. However, it has been shown that L-NAME both enhances (Noda et al., 1995a) and abolishes (Johansson et al., 1997, 1998) PCP-induced behavioural effects. Considering these conflicting data further studies are required to elucidate the participation of NO in the effects produced by PCP. Therefore, in the present study we investigated the effect of L-NAME on PCP-induced behaviour and c-*fos* expression. Increased expression of the protein product of the immediate early gene c-*fos* is now widely recognised as a reliable technique to identify neuronal populations of metabolically activated brain regions (Sagar et al., 1988; Hughes and Dragunov, 1995; Herrera and Robertson, 1996). Some of the data reported in the present paper have been presented in abstract form (Bujas-Bobanovic et al., 1998b).

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats (Charles River, Quebec, Canada) weighing 250–300 g were used in this study. They arrived at the animal facilities at least 5 days prior to the start of the experiments. Rats were housed in groups of two on a 12-h dark–light cycle (lights on at 7:00 h) at 22°C. Food and water were available ad libitum during the time the animals were in their home cages.

2.2. Materials

Phencyclidine hydrochloride was generously donated by the Bureau of Drug Surveillance (Ottawa, Canada). L-NAME was purchased from Sigma (St. Louis, MO). Drugs were freshly dissolved in 0.9% saline before each experiment and were administered intraperitoneally (i.p.) in a volume of 0.1 ml/100 g body weight.

2.3. Experimental schedule

On experimental days, the animals were weighed and placed individually in a transparent acrylic cage. They were allowed to habituate for 1 h. Rats were injected with saline or L-NAME (5, 10, 25 mg/kg) 15 min before PCP (5 mg/kg) or saline. The dose of 5 mg/kg of PCP was chosen according to dose–response study performed previously in our laboratory (Bujas-Bobanovic et al., 1998a) and produces maximal behavioural response with no sign of motor co-ordination impairment. The observation period started immediately after the last injection. Behaviour was observed for 1 h, according to the method of previous reports (Sturgeon et al., 1979; Castellani and Adams, 1981). No rating scales were used since distinct behavioural patterns were observed following different drug treatments. The observer was blind to the treatment given to each animal until the experiment was concluded. Rats were randomly allocated to treatment groups and used only once. All experiments were carried out between 08:00 and 14:00 h.

2.4. Immunocytochemistry

At 1 h following PCP or saline injection, rats were deeply anaesthetised with sodium pentobarbital (> 70 mg/kg) and perfused transcardially with 60 ml of 0.1 M phosphate-buffered saline (PBS), followed by 120 ml of fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). Brains were removed, postfixed for 48 h and cut sagittaly on a Vibratome to a thickness of 50 μm. Free-floating sections were processed for c-*fos* immunocytochemistry. Sections were incubated for 20 min in 1% H_2O_2 to inactivate endogenous peroxidase activity and reduce non-specific staining. Sections were washed three times (10 min per wash) in 0.01 M PBS containing 0.2% Triton X-100 (PBS_x), then incubated on a rocking table with c-*fos* antibody for 48 h at 4°C. C-*fos* polyclonal antibody (Santa Cruz, 1:20,000) has been raised in rabbit against residues 3–16 of the N-terminal region of the Fos protein. Sections were washed again three times in PBS_x prior to being incubated with the biotinylated goat anti-rabbit secondary antibodies (Vector Laboratories, 1:200) for at least 1 h at room temperature. Immunostaining was visualised using the standard avidin–biotin technique. After again washing three times in PBS_x, the sections were incubated with the avidin-biotinylated horseradish peroxidase complex (VECTASTAIN Elite ABC Kit, Vector Laboratories) for at least 1 h, rinsed three times in PBS_x and placed in the chromogen 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma—for every DAB tablet: 20 μl glucose oxidase, 40 μl ammonium chloride and 160 μl D(+) glucose was added) for 15 min. The sections were again washed three times in PBS_x, mounted on gelatinised slides and left overnight to dry. The mounted sections were then dehydrated and defatted through a graded alcohol series to xylene and were coverslipped using entellan (E. Merck, Germany). No immunoreactivity was observed when the primary antibody was omitted.

2.5. Immunocytochemical analysis

Brain sections were analysed under a light microscope. Co-ordinates of sagittal planes and limits of the various

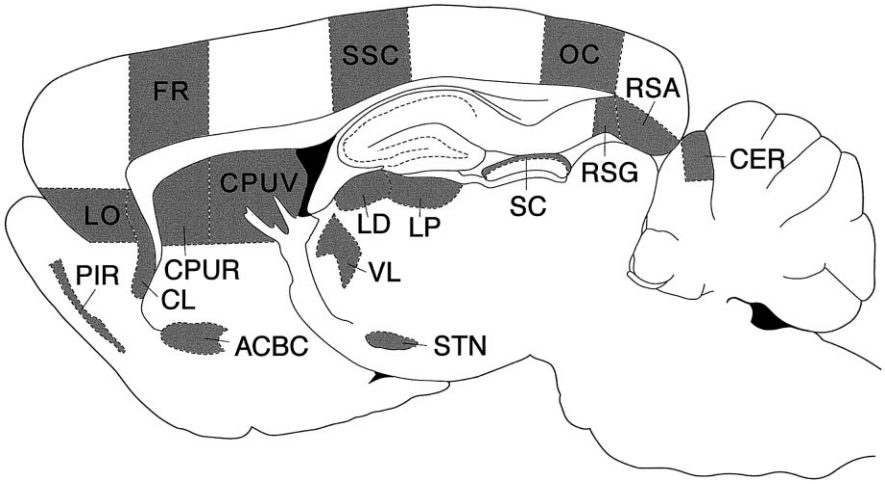

Fig. 1. Schematic drawing adapted from Paxinos and Watson (1998) showing brain regions chosen for quantitative analysis (grey areas). PIR, piriform cortex; CL, claustrum; LO, lateral orbital cortex; FR, frontal cortex; SSC, somatosensory cortex; OC, occipital cortex; RSA, retrosplenial agranular cortex; RSG, retrosplenial granular cortex; CPUR, rostral part of the caudate putamen; CPUV, periventricular portion of the caudate putamen; ACBC, nucleus accumbens core; VL, ventrolateral thalamic nucleus; LD, laterodorsal thalamic nucleus; LP, lateral posterior thalamic nucleus; STN, subthalamic nucleus; SC, zonal layer superior colliculus; CER, cerebellar lobulus 5.

structures analysed were defined according to the atlas of Paxinos and Watson (1998). In pilot studies sections were examined throughout the brain to determine the areas with the most prominent c-*fos* immunostaining. On this basis, one section, at 2.4 mm lateral to the midline, was selected for counting the number of fos positive nuclei through each of 17 activated brain areas: piriform cortex; claustrum; lateral orbital cortex; frontal cortex; somatosensory cortex; occipital cortex; retrosplenial agranular cortex; retrosplenial granular cortex; rostral part of the caudate putamen; periventricular portion of the caudate putamen; nucleus accumbens core; ventrolateral thalamic nucleus; laterodorsal thalamic nucleus; lateral posterior thalamic nucleus; subthalamic nucleus; zonal layer superior colliculus; cerebellar lobulus 5 (Fig. 1). In addition, one section, at 4.2 mm lateral to the midline, was selected to count the number of fos positive nuclei in the entorhinal cortex. Regions were analysed in one hemisphere. Quantification of fos positive nuclei was performed by counting immunopositive cells plotted at either a $\times 4$ or $\times 10$ magnification. Sections were scanned into the computer using Adobe Photoshop 4.0 (Adobe Systems, San Jose, CA, USA). Cell counts were made with the help of a computer-assisted imaging analysis system (analysis performed on a Macintosh computer using the public domain NIH Image program developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The density slice option was used to facilitate the counting. The minimum and maximum particle size to be analysed was set to 5 and 55 pixels, respectively. The gray level range of Fos positive nuclei was calibrated for all sections through a given area, as described previously (Auger and Blaustein, 1995). Sections were carefully matched across animals under a microscope according to the morphological structures. Analyses were conducted in random order by an observer blind with respect to treatment.

2.6. Statistical analysis

The results are presented as the mean and S.E.M. for each experimental group. The c-*fos* data were analysed by two-factor (PCP $\times$ L-NAME) analysis of variance (ANOVA). The Bonferroni test for multiple group comparison was used for post hoc analyses. Differences revealed with both the ANOVAs and the post hoc tests were considered significant at $P < 0.05$. Data analysis was done using SPSS Base 8.0 for Windows.

3. Results

PCP (5 mg/kg) alone induced a characteristic behavioural response with a remarkable hyperactivity and stereotyped behaviours, without apparent ataxia. Animals were moving over a large area of the cage and the activity was intermittent and emitted at a moderate-rapid rate. Stereotyped behaviours included rapid rate and continuous head weaving, turning and backpedalling. All behavioural effects occurred within a few minutes and reached a maximum between 20 and 30 min after injection. Although L-NAME by itself failed to produce any behavioural effects, pretreatment with L-NAME (5, 10 or 25 mg/kg) potentiated PCP-induced behaviour, i.e. animals displayed severe ataxia which interfered with the rats' ability to exhibit hyperactivity and stereotyped behaviours. Animals could not support their own weight but could only crawl, pushing themselves forward by their legs, i.e. their be-

haviour resembled that produced by high dose of PCP, as reported previously (Sturgeon et al., 1979; Bujas-Bobanovic et al., 1998a).

PCP (5 mg/kg) induced widespread expression of c-*fos* in neurones in many brain regions, as compared with saline-treated animals (Table 1). Moreover, PCP induced regionally different effects on c-*fos* expression, suggesting regionally different patterns of neuronal activity. The most prominent immunostaining was observed in the cortical neurones, followed by claustrum, nucleus accumbens core, thalamic and subthalamic nuclei, superior colliculus and cerebellum. L-NAME increased PCP-induced c-*fos* expression in many brain regions and changed the regional distribution to the pattern observed previously with higher

Table 1
Effect of L-NAME (5, 10, 25 mg/kg) on PCP (5 mg/kg)-induced c-*fos* expression

(a)

Brain region	PCP	L-NAME	PCP × L-NAME
PIR	$F(1,24) = 176.1,\ P < 0.0001$	$F(3,24) = 5.1,\ P < 0.01$	$F(3,24) = 3.1,\ P < 0.05$
CL	$F(1,24) = 280.6,\ P < 0.0001$	$F(3,24) = 4.0,\ P < 0.05$	$F(3,24) = 1.5,\ P = 0.2$
LO	$F(1,24) = 316.8,\ P < 0.0001$	$F(3,24) = 4.4,\ P < 0.05$	$F(3,24) = 3.1,\ P < 0.05$
FR	$F(1,24) = 264.1,\ P < 0.0001$	$F(3,24) = 12.5,\ P < 0.0001$	$F(3,24) = 8.4,\ P < 0.005$
SSC	$F(1,24) = 499.7,\ P < 0.0001$	$F(3,24) = 10.0,\ P < 0.0001$	$F(3,24) = 3.3,\ P < 0.05$
OC	$F(1,24) = 146.5,\ P < 0.0001$	$F(3,24) = 11.2,\ P < 0.0001$	$F(3,24) = 0.2,\ P = 0.9$
RSA	$F(1,24) = 486.9,\ P < 0.0001$	$F(3,24) = 1.0,\ P = 0.4$	$F(3,24) = 3.7,\ P < 0.05$
RSG	$F(1,24) = 238.8,\ P < 0.0001$	$F(3,24) = 14.4,\ P < 0.0001$	$F(3,24) = 6.9,\ P < 0.005$
ENT	$F(1,24) = 232.5,\ P < 0.0001$	$F(3,24) = 28.9,\ P < 0.0001$	$F(3,24) = 20.4,\ P < 0.0001$
CPUR	$F(1,24) = 48.6,\ P < 0.0001$	$F(3,24) = 7.0,\ P < 0.005$	$F(3,24) = 7.1,\ P < 0.005$
CPUV	$F(1,24) = 42.5,\ P < 0.0001$	$F(3,24) = 8.1,\ P < 0.005$	$F(3,24) = 1.7,\ P = 0.2$
ACBC	$F(1,24) = 90.3,\ P < 0.0001$	$F(3,24) = 1.7,\ P = 0.2$	$F(3,24) = 2.5,\ P = 0.1$
VL	$F(1,24) = 123.3,\ P < 0.0001$	$F(3,24) = 1.9,\ P = 0.2$	$F(3,24) = 2.1,\ P = 0.1$
LD	$F(1,24) = 188.9,\ P < 0.0001$	$F(3,24) = 8.5,\ P < 0.005$	$F(3,24) = 7.4,\ P < 0.005$
LP	$F(1,24) = 204.3,\ P < 0.0001$	$F(3,24) = 7.7,\ P < 0.005$	$F(3,24) = 6.6,\ P < 0.005$
STN	$F(1,24) = 200.3,\ P < 0.0001$	$F(3,24) = 8.4,\ P < 0.005$	$F(3,24) = 5.9,\ P < 0.005$
SC	$F(1,24) = 160.6,\ P < 0.0001$	$F(3,24) = 3.6,\ P < 0.05$	$F(3,24) = 3.2,\ P < 0.05$
CER	$F(1,24) = 187.7,\ P < 0.0001$	$F(3,24) = 16.1,\ P < 0.0001$	$F(3,24) = 7.4,\ P < 0.005$

(b)

Brain region	S + S	S + PCP	L-NAME 5 + S	L-NAME 5 + PCP	L-NAME 10 + S	L-NAME 10 + PCP	L-NAME 25 + S	L-NAME 25 + PCP
PIR	23 ± 7	211 ± 21^{d}	62 ± 13	184 ± 12	87 ± 11^{a}	258 ± 25	83 ± 11	189 ± 17
CL	9 ± 4	103 ± 8^{d}	9 ± 3	143 ± 11^{e}	21 ± 7	136 ± 14	33 ± 6	143 ± 16^{e}
LO	18 ± 9	456 ± 30^{d}	58 ± 9	381 ± 22	125 ± 19	506 ± 57	113 ± 17	389 ± 29
FR	20 ± 2	207 ± 37^{c}	39 ± 9	519 ± 52^{g}	58 ± 10	500 ± 63^{g}	58 ± 12	539 ± 35^{g}
SSC	19 ± 2	535 ± 30^{d}	46 ± 4	753 ± 66^{f}	138 ± 15	835 ± 32^{g}	149 ± 33	693 ± 67
OC	57 ± 14	613 ± 106^{d}	318 ± 38	963 ± 56^{e}	365 ± 62^{a}	969 ± 34^{e}	381 ± 64^{a}	1019 ± 125^{f}
RSA	20 ± 6	414 ± 30^{d}	41 ± 12	397 ± 21	102 ± 22	396 ± 35	102 ± 11	371 ± 15
RSG	8 ± 2	84 ± 7^{d}	17 ± 5	189 ± 9^{g}	36 ± 14	170 ± 13^{f}	37 ± 10	219 ± 27^{g}
ENT	27 ± 12	144 ± 10	63 ± 4	662 ± 70^{g}	67 ± 3	586 ± 66^{g}	101 ± 10	972 ± 97^{g}
CPUR	2 ± 1	7 ± 1	2 ± 0	60 ± 9^{f}	2 ± 1	90 ± 24^{g}	16 ± 3	50 ± 4^{e}
CPUV	19 ± 3	76 ± 11^{a}	61 ± 9	155 ± 21^{e}	70 ± 11	173 ± 23^{f}	70 ± 16	111 ± 23
ACBC	13 ± 6	81 ± 9^{c}	13 ± 5	102 ± 25	13 ± 3	136 ± 22^{e}	25 ± 5	84 ± 4
VL	8 ± 4	69 ± 21^{c}	6 ± 1	114 ± 22	8 ± 2	118 ± 9^{e}	17 ± 2	97 ± 5
LD	4 ± 1	153 ± 37^{b}	9 ± 2	471 ± 47^{g}	17 ± 2	425 ± 49^{g}	22 ± 4	428 ± 69^{g}
LP	7 ± 1	161 ± 39^{c}	16 ± 4	403 ± 49^{g}	16 ± 3	418 ± 51^{g}	20 ± 4	406 ± 46^{g}
STN	3 ± 1	44 ± 12^{c}	5 ± 2	98 ± 10^{f}	9 ± 1	112 ± 17^{g}	9 ± 3	109 ± 4^{g}
SC	3 ± 1	57 ± 18^{c}	4 ± 1	116 ± 15^{f}	5 ± 2	106 ± 11^{e}	6 ± 1	94 ± 10
CER	4 ± 1	68 ± 18^{c}	22 ± 4	167 ± 12^{g}	34 ± 6	228 ± 22^{g}	30 ± 8	165 ± 22^{g}

(a) Two-factorial ANOVA.
(b) Data are expressed as mean of positive fos-immunoreactive nuclei ± S.E.M. ($n = 4$ per group).
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.005$.
[d] $P < 0.0001$ vs. S + S.
[e] $P < 0.05$.
[f] $P < 0.005$.
[g] $P < 0.0001$ vs. S + PCP.

doses of PCP (Bujas-Bobanovic et al., 1998a). The most prominent enhancement was observed in the cortical neurones. While L-NAME, at all doses tested, was effective in the frontal, occipital and retrosplenial granular cortex, L-NAME 5 and 10 mg/kg also significantly increased PCP-induced c-*fos* expression in the somatosensory cortex. In addition, L-NAME 5 and 10 mg/kg increased PCP-induced c-*fos* expression in the superior colliculus, periventricular portion of the caudate putamen, subthalamic nucleus, lateral posterior and laterodorsal thalamic nuclei and in the cerebellar granule cell layer. L-NAME 25 mg/kg increased PCP-induced c-*fos* expression in the subthalamic nucleus, lateral posterior and laterodorsal thalamic nuclei and cerebellum. The most interesting finding was observed in the entorhinal cortex and rostral part of the caudate putamen. As shown in Fig. 2 and Table 1, PCP 5 mg/kg induced low c-*fos* expression in the entorhinal cortex, with almost no expression in the rostral part of caudate putamen. However, animals pretreated with L-NAME (5–25 mg/kg) showed a marked activation in these brain areas. On the other hand, L-NAME, at all doses tested, failed to affect PCP-induced c-*fos* expression in the lateral orbital, piriform and retrosplenial agranular cortex. Although L-NAME by itself induced c-*fos* expression in several brain regions, the only significant difference was observed in the piriform cortex between the groups given saline + saline and L-NAME 10 mg/kg + saline and in the occipital cortex between the groups given saline + saline and the groups given L-NAME 10 mg/kg + saline and L-NAME 25 mg/kg + saline.

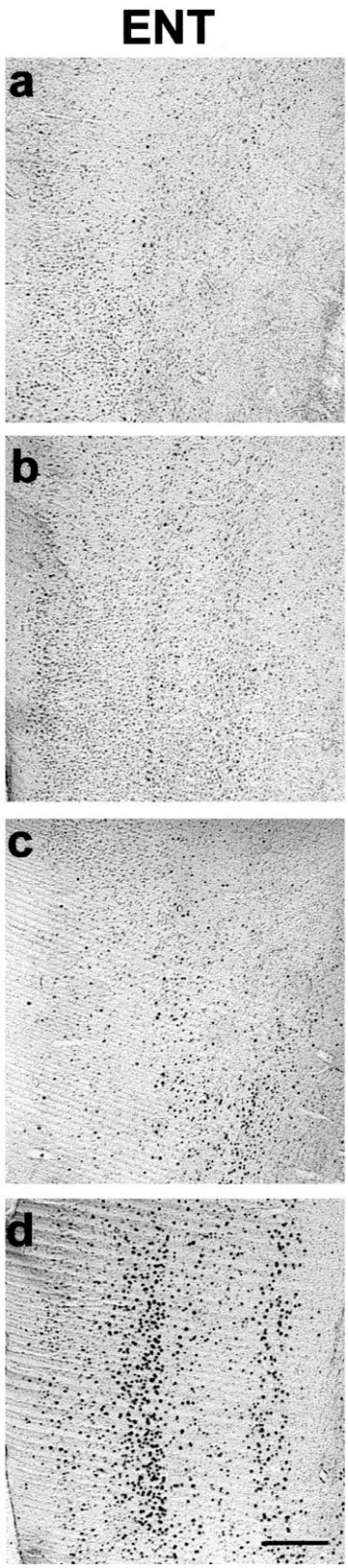

Fig. 2. The effect of L-NAME (10 mg/kg) on PCP (5 mg/kg)-induced c-*fos* expression in the entorhinal cortex. (a) Saline + saline; (b) L-NAME + saline, (c) saline + PCP, (d) L-NAME + PCP. Bar = 143 μm.

4. Discussion

In agreement with previous findings (Sturgeon et al., 1979; Castellani and Adams, 1981; Yang et al., 1991; Toru et al., 1994; Steinpreis, 1996), the present data indicate that PCP induces a complex behavioural syndrome with increased locomotor activity and stereotyped behaviours consisting of head weaving, turning and backpedalling. Although PCP activates many brain regions, the most prominent effect was observed in the cortical regions. This is particularly interesting because the cerebral cortex has been considered to be a therapeutic target of antipsychotic drugs (Lidow et al., 1998). Recent evidence from brain imaging studies has also indicated cortical involvement in positive, negative and cognitive symptoms of schizophrenia (Dolan et al., 1995; Silbersweig and Stern, 1996; Okubo et al., 1997). The overall distribution patterns of c-*fos* induced by PCP appear consistent with those previously reported for c-*fos* mRNA and fos protein, following administration of high doses of PCP (Näkki et al., 1996; Sharp, 1997). These patterns are distinct from those following injection of other schizophrenomimetic dopamine agonists such as amphetamine and cocaine (Graybiel et al., 1990; Umino et al., 1995), but resemble those induced by other NMDA receptor antagonists such as ketamine and MK-801 (Gass et al., 1993; Duncan et al., 1998). This may

suggest that PCP-induction of c-*fos* may be primarily due to the reduced glutamatergic neurotransmission. However, c-*fos* distribution following PCP administration does not correspond completely to the distribution of NMDA receptors (Thornberg and Saklad, 1996). This may be explained by the fact that, in addition to blocking NMDA receptors, PCP also interacts with many other neurotransmitter systems. Therefore, multiple receptors and different pathways, other than glutamatergic, may be involved in the activation of c-*fos*. For instance, the metabolic activation induced by PCP may result from functional antagonism of inhibitory neural processes, such as blocking NMDA receptors on γ-aminobutyric acid (GABA)-containing neurones, thus reducing inhibitory tone in the brain. Pre-treatment with L-NAME potentiated behavioural effects of PCP and increased PCP-induced c-*fos* expression. Thus, we confirmed the data of Noda et al. (1995a) showing that NOS inhibitors enhance PCP-induced behaviours in mice. The present results are also consistent with our previous study (Bujas-Bobanovic et al., 2000) showing that sodium nitroprusside, a NO donor, reverses both PCP-induced behaviour and neuronal activation. However, our results are in contrast to those of Johansson et al. (1997, 1998, 1999) suggesting that NOS inhibition blocks PCP-induced effects. There is at least one explanation for these different results. To analyse thoroughly the effects of drugs on PCP-induced behaviour, it is necessary to analyse all three behaviours (locomotor activity, stereotyped behaviour, ataxia) simultaneously. However, Johansson et al. monitored only the locomotor activity and stereotyped behaviour. We observed that ataxia, produced by high doses of PCP (Bujas-Bobanovic et al., 1998a), or by low dose of PCP in combination with L-NAME (this study), impairs the ability of animals to execute hyperlocomotor activity and stereotyped behaviours. It is also important to point out that acute injection of L-NAME, at doses higher than those used in our study, produces a significant decrease in the spontaneous locomotor activity (Sandi et al., 1995; Yamada et al., 1995). Therefore, studies that rely exclusively on only locomotor activity and/or stereotyped behaviours may result in misleading conclusions. The exact mechanism by which L-NAME enhanced PCP-induced effects is not clear. Glutamate, acting through NMDA receptors, appears to be the principal activation signal for neuronal NO production (Ignarro and Murad, 1995; Dawson and Dawson, 1996; Szabo, 1996). Therefore, PCP blockade of NMDA receptors might lead to a decrease in NO levels, an effect that is synergistic with the action of NOS inhibitors. Indeed, several authors have reported that PCP is an effective inhibitor of neuronal NOS (Osawa and Davila, 1993; Chetty et al., 1995) and that NOS activity is significantly decreased by acute PCP treatment (Noda et al., 1996). Moreover, it has been repeatedly shown that PCP, and its analogue, MK-801, have an effect similar to that of NOS inhibitors. For example, hippocampal long-term potentiation is inhibited by NMDA receptor antagonists and by NOS inhibitors (O'Dell et al., 1991; Schuman and Madison, 1991). These antagonists have also been found to protect against NMDA-induced neurotoxicity (Dawson et al., 1993; Ayata et al., 1997; Sattler et al., 1999). They also block NMDA-mediated convulsions (Nakamura et al., 1995), and impair learning and memory in several animal models (Chapman et al., 1992; Böhme et al., 1993; Hölscher et al., 1995; Yamada et al., 1995; Harder et al., 1998; Ingram et al., 1998; Meyer et al., 1998; Zou et al., 1998). However, there is also evidence that inhibition of NOS does not impair learning or prevent the induction of long-term potentiation and NMDA-induced convulsions in animals (Buisson et al., 1993; Bannerman et al., 1994a,b).

It is well known that c-*fos* can be induced in response to various stimuli (for review see Morgan and Curran, 1991). It has been suggested that its triggering requires a high level of synaptic activity (Sgambato et al., 1997). Therefore, the additive effects of L-NAME and PCP on synaptic activity may explain why L-NAME by itself only weakly induces c-*fos* in many regions of brain where it potentiates the effects of PCP. We observed that L-NAME-induced increases in c-*fos* expression were widely distributed beyond the areas containing NO. However, NO has been implicated in the release of acetylcholine, norepinephrine, dopamine, glutamate and GABA (Szabo, 1996). Therefore, involvement of neurotransmitter systems, other than NO, might explain such a widespread c-*fos* induction. In the previous study (Bujas-Bobanovic et al., 1998a), we showed that striatal neurones were mostly unaffected following PCP 2.5–10 mg/kg. Although electrophysiological studies have shown that low doses of PCP (1–5 mg/kg) increase striatal neuron firing concomitant with behavioural activation (White et al., 1995), the activity level was probably not sufficient enough to induce c-*fos* expression. However, we showed that striatal neurones were activated following high doses of PCP (25–50 mg/kg), (Bujas-Bobanovic et al., 1998a) or low doses of PCP (5 mg/kg) in L-NAME-pretreated animals (this study). It has been reported that the electrical stimulation of the motor cortex induces c-*fos* expression in the striatum (Sgambato et al., 1997) and its distribution pattern corresponds to the pattern we observed following high doses of PCP (Bujas-Bobanovic et al., 1998a) or low dose of PCP in L-NAME-pretreated animals (this study). Since these animals showed severe motor impairment, we propose that c-*fos* induction in the striatum is mediated by strong synaptic activity in the motor cerebral cortex. In addition to the striatum, we showed that the entorhinal cortex is also activated only when animals were injected with high doses of PCP (Bujas-Bobanovic et al., 1998a) or low doses of PCP in L-NAME-pretreated animals (this study). Interestingly, both morphological and functional abnormalities have been reported in the entorhinal cortex of patients with schizophrenia (Jakob and Beckmann, 1986; Arnold et al., 1991; Friston et al., 1992), suggesting that

the entorhinal abnormality is of major significance in the pathophysiology of schizophrenia (Roberts, 1991).

In conclusion, the present study shows that L-NAME potentiates both PCP-induced behaviour and PCP-induced neuronal activation, as monitored by c-*fos* expression. Although L-NAME is a non-specific inhibitor of neuronal NOS (nNOS), many studies have shown similar effects between L-NAME and 7-nitroindazole, a selective nNOS inhibitor, in different animal models (e.g. Noda et al., 1995a,b; Itzhak and Ali, 1996; Taraska and Finnegan, 1997; Ingram et al., 1998; Meyer et al., 1998). Therefore, it is unlikely that the effects of L-NAME on PCP-induced changes found in this study can be due to a nonspecific effect, secondary to the action of L-NAME on other NOS isoforms.

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