Activation of Thalamocortical Networks by the *N*-methyl-D-aspartate Receptor Antagonist Phencyclidine: Reversal by Clozapine

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Background: Noncompetitive *N*-methyl-D-aspartate receptor antagonists are widely used as pharmacological models of schizophrenia. Their neurobiological actions are still poorly understood, although the prefrontal cortex (PFC) appears as a key target area.

Methods: We examined the effect of phencyclidine (PCP) on neuronal activity of the mediodorsal (MD) and centromedial (CM) thalamic nuclei, reciprocally connected with the PFC, using extracellular recordings ($n = 50$ neurons from 35 Wistar rats) and *c-fos* expression.

Results: Phencyclidine (.25 mg/kg intravenous [IV]) markedly disorganized the activity of MD/CM neurons, increasing (424%) and decreasing (41%) the activity of 57% and 20% of the recorded neurons, respectively (23% remained unaffected). Phencyclidine reduced delta oscillations (.15–4 Hz) as assessed by recording local field potentials. The subsequent clozapine administration (1 mg/kg IV) reversed PCP effects on neuronal discharge and delta oscillations. Double in situ hybridization experiments revealed that PCP (10 mg/kg intraperitoneal [IP]) markedly increased c-*fos* expression in glutamatergic neurons of several cortical areas (prefrontal, somatosensory, retrosplenial, entorhinal) and in thalamic nuclei, including MD/CM. Phencyclidine also increased c-*fos* expression in the amygdala; yet, it had a small effect in the hippocampus. Phencyclidine did not increase c-*fos* expression in gamma-aminobutyric acidergic cells except in hippocampus, amygdala, somatosensory, and retrosplenial cortices. Clozapine (5 mg/kg IP) had no effect by itself but significantly prevented PCP-induced c-*fos* expression.

Conclusions: Phencyclidine likely exerts its psychotomimetic action by increasing excitatory neurotransmission in thalamo-cortico-thalamic networks involving, among others, PFC, retrosplenial, and somatosensory cortices. The antipsychotic action of clozapine includes, among other actions, an attenuation of the neuronal hyperactivity in thalamocortical networks.

Key Words: Antipsychotics, delta oscillations, GABAergic interneurons, glutamate, prefrontal cortex, pyramidal neurons, thalamus

Noncompetitive *N*-methyl-D-aspartate receptor (NMDA-R) antagonists are extensively used as pharmacological models of schizophrenia due to their ability to evoke schizophrenia symptoms in healthy individuals and to aggravate them in schizophrenic patients (1). These agents also induce behavioral, cognitive, and sensory abnormalities in experimental animals that resemble the human illness (2–5) and are sensitive to antipsychotic drugs (1,6). Nevertheless, their neurobiological basis of action is still poorly understood. Neuroimaging studies indicate that ketamine increases metabolic activity in prefrontal cortex (PFC) of human volunteers in parallel with the emergence of psychotic symptoms (7). Likewise, dizocilpine (MK-801) or phencyclidine (PCP) increases neuronal activity (8–10) and decreases cortical synchrony in rat PFC (10). Both effects are reversed by antipsychotic drugs, which suggests a direct relationship to their therapeutic action (10). These NMDA-R antagonist actions are accompanied by increased PFC release of glutamate (11–14), dopamine (15–17), serotonin (12,14,18–20) and acetylcholine (21). Overall, these effects likely mediate the deleterious effects of NMDA-R antagonists on cognitive processes (1,9), given the involvement of the PFC in higher brain functions (22,23) altered in schizophrenia.

Despite these remarkable actions in PFC, it is still unclear whether NMDA-R antagonists primarily affect PFC or whether other cortical and subcortical areas reciprocally connected with the PFC (24) are also involved. The opposite effects of MK-801 on the activity of putative pyramidal and gamma-aminobutyric acidergic (GABAergic) neurons (increase and decrease, respectively) led to proposal of a preferential blockade of NMDA-R on cortical GABAergic interneurons and a subsequent disinhibition of pyramidal neurons (9,25). However, local PCP or MK-801 administration in medial PFC reduced the discharge of putative pyramidal neurons (8,26), and systemic, but not intra-PFC, administration of NMDA-R antagonists increased neurotransmitter release in PFC (14,20), suggesting that NMDA-R blockade in other brain areas may also contribute to increased PFC activity.

We previously reported that PCP induced a marked PFC hyperactivity in parallel with increased c-*fos* expression in this cortical area (10). Pilot observations also indicated an increased c-*fos* expression in the mediodorsal (MD) and centromedial (CM) thalamic nuclei, reciprocally connected with PFC (24,27). Here, we examined the effect of PCP on neuronal activity in MD/CM. Likewise, we extend previous observations by performing an extensive quantitative study of the effect of PCP on c-*fos* expression in glutamatergic and GABAergic neurons in rat brain, paying a special attention to thalamo-cortico-thalamic networks. Finally, we examined the ability of the atypical antipsychotic clozapine (CLZ) to antagonize PCP-induced changes in both experimental paradigms.

Methods and Materials

Animals and Treatments

Male albino Wistar rats weighing 250 g to 320 g were used (Iffa Credo, Lyon, France). Animal care followed European Union regula-

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tions (Official Journal of the European Communities L358/1, December 18, 1986) and was approved by the Institutional Animal Care and Use Committee. The PCP and CLZ were from Sigma/RBI (Natick, Massachusetts). In electrophysiological experiments, rats were treated intravenously (IV) with PCP (.25 mg/kg) followed by CLZ (1 mg/kg). In c-*fos* experiments, four groups of rats were administered intraperitoneal (IP) saline (SAL) plus SAL, SAL plus PCP (10 mg/kg), CLZ (5 mg/kg) plus SAL, and CLZ (5 mg/kg) plus PCP (10 mg/kg) (SAL + SAL, SAL + PCP, CLZ + SAL and CLZ + PCP, respectively). Time between injections was 30 minutes, and rats were killed 1 hour after the second injection by anesthetic overdose. The brains were rapidly removed, frozen on dry ice, and stored at −20°C.

Electrophysiology: Single Unit and Local Field Potential Recordings

Electrophysiological procedures were essentially as described in Kargieman *et al.* (10). We performed local field potential (LFP) and single-unit extracellular recordings of thalamic neurons in anesthetized rats (chloral hydrate 400 mg/kg IP, followed by 50 to 70 mg/kg/hour using a perfusion pump). Neurons in the CM and MD thalamic nuclei (Figure 1) were recorded with glass micropipettes filled with 2% pontamine sky blue (Avocado Research Chemicals Ltd, Lancaster, United Kingdom) in 2 mol/L sodium chloride. Descents were carried out at anteroposterior −2.5, left −.6 to .8, dorsoventral −4.7 to −6.2 below brain surface (coordinates in mm) (28). The identification of thalamic neurons and burst-firing analysis were carried out according to the criteria of Sittig and Davidowa (29). Neurons were considered glutamatergic thalamic neurons if they exhibited burst firing with two or more spikes having an interspike interval of ≤ 4 milliseconds (Figure 1A). After recording electrical activity, pontamine sky blue was iontophoretically ejected (20 μA cathodal current, 20 minutes) to verify the recording site. Once experimental procedures were completed, animals were killed by an anesthetic overdose and the brains rapidly removed and frozen on dry ice before being sectioned (50 μm) with a cryostat in coronal planes. Brain sections were stained with neutral red, according to standard procedures, to verify the recording site.

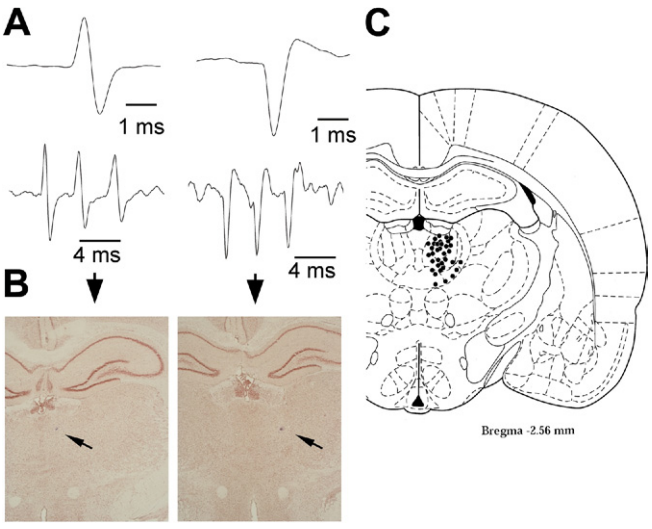

Figure 1. Extracellular recording of thalamic neurons. **(A)** Representative spikes and firing pattern of thalamic neurons. **(B)** Location of the neurons displayed in **(A)**. **(C)** Schematic representation of the recording sites of all neurons. Reprinted from (67), with permission from Elsevier, copyright 1997.

Table 1. Effects of PCP Administration on Burst Firing of Thalamic Neurons

	Group	Basal	PCP (.25 mg/kg)
Firing Rate (spikes/sec)	A	1.7 ± .2	4.0 ± 1.0[a]
	E	1.9 ± .3	6.3 ± 1.6[a]
	I	1.3 ± .4	.5 ± .2[a]
% of Spikes Fired in Burst	A	36 ± 3	28 ± 4
	E	41 ± 4	28 ± 6[b]
	I	29 ± 8	17 ± 10
Spikes in Bursts (2 min)	A	87 ± 14	99 ± 22
	E	104 ± 20	139 ± 33
	I	57 ± 24	7 ± 5
Spikes per Burst	A	2.63 ± .07 (32)	2.66 ± .09 (28)
	E	2.68 ± .08 (19)	2.83 ± .12 (16)
	I	2.59 ± .19 (6)	2.37 ± .23 (5)
Burst Episodes (2 min)	A	31 ± 5	36 ± 9
	E	37 ± 7	50 ± 13
	I	20 ± 8	3 ± 2
Burst Duration (msec)	A	4.99 ± .18 (32)	4.95 ± .24 (28)
	E	5.12 ± .23 (19)	5.42 ± .28 (16)
	I	4.91 ± .57 (6)	3.95 ± .47 (5)
Interspike Interval Within Bursts (msec)	A	3.15 ± .05 (32)	3.08 ± .06 (28)
	E	3.14 ± .06 (19)	3.08 ± .08 (16)
	I	3.19 ± .16 (6)	3.03 ± .23 (5)
N	A	35	35
	E	20	20
	I	7	7

A, all neurons; E, excited neurons; I, inhibited neurons; PCP, phencyclidine.
[a] $p < .02$ vs. baseline.
[b] $p < .04$ vs. baseline.

In Situ Hybridization Histochemistry

Double in situ hybridization experiments were essentially conducted as in Kargieman *et al.* (10). Briefly, radioactive oligonucleotides were used to label *c-fos* messenger RNA, whereas the neuronal phenotypes (glutamatergic or GABAergic) were identified with nonradioactive (digoxigenin) oligonucleotides.

The oligonucleotide probes used were as follows: c-*fos*, complementary to bases 131–178 (GenBank accession no. NM_022197); vesicular glutamate transporter 1 (vGluT1) (glutamatergic cell marker), complementary to bases 127–172 and 1756–1800 (GenBank accession no. U07609); glutamic acid decarboxylase (GAD) (GAD65 and GAD67 isoforms, to label GABAergic cells) complementary to bases 159–213 and 514–558 (GenBank accession no. NM_012563) and to bases 191–235 and 1600–1653 (GenBank accession no. NM_017007). Labeling of the probes, tissue sectioning, and in situ hybridization procedures were carried out as described previously (30,31). Despite thalamic neurons mainly express vesicular glutamate transporter 2, vGluT1 is present in lower density (32,33) and was clearly detectable in our experimental conditions in thalamic cells. For this reason, we identified excitatory neurons in cortical and thalamic areas with vGluT1 messenger RNA.

Data Analysis

Changes in the firing rate or the proportion of burst firing in thalamic neurons were analyzed using analysis of variance (ANOVA)

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