The Hallucinogen DOI Reduces Low-Frequency Oscillations in Rat Prefrontal Cortex: Reversal by Antipsychotic Drugs

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Background: Perceptual and psychic alterations and thought disorder are fundamental elements of schizophrenia symptoms, a pathology associated with an abnormal macro- and microcircuitry of several brain areas including the prefrontal cortex (PFC). Alterations in information processing in PFC may partly underlie schizophrenia symptoms.

Methods: The 5-$HT_{2A/2C}$ agonist DOI and antipsychotic drugs were administered to anesthetized rats. Single unit and local field potential (LFP) extracellular recordings were made in medial PFC (mPFC). Electrolytic lesions were performed in the thalamic nuclei.

Results: DOI markedly disrupts cellular and network activity in rat PFC. DOI altered pyramidal discharge in mPFC (39% excited, 27% inhibited, 34% unaffected; n = 51). In all instances, DOI concurrently reduced low-frequency oscillations (.3–4 Hz; power spectrum: .25 ± .02 and .14 ± .01 μV^2 in basal conditions and after 50–300 μg/kg intravenous (IV) DOI, respectively; n = 51). Moreover, DOI disrupted the temporal association between the active phase of LFP and pyramidal discharge. Both effects were reversed by M100907 (5-HT_{2A} receptor antagonist) and were not attenuated by thalamic lesions, supporting an intracortical origin of the effects of DOI. The reduction in low-frequency oscillations induced by DOI was significantly reversed by the antipsychotic drugs haloperidol (.1–.2 mg/kg IV) and clozapine (1 mg/kg IV).

Conclusions: DOI disorganizes network activity in PFC, reducing low-frequency oscillations and desynchronizing pyramidal discharge from active phases of LFP. These effects may underlie DOI's psychotomimetic action. The reversal by clozapine and haloperidol indicates that antipsychotic drugs may reduce psychotic symptoms by normalizing an altered PFC function.

Key Words: 5-HT_{2A} receptors, EEG, local field potential, psychotomimetic agents, pyramidal neurons, schizophrenia

Schizophrenia is associated with anatomic, cellular, and neurochemical alterations of the prefrontal cortex (PFC) among other brain structures (1–5). The PFC is critically involved in many higher brain functions, including cognition, attention, and behavioral control (6,7) which are altered in schizophrenia patients (8).

A mesocortical dopaminergic hypoactivity is associated with the cognitive deficits and negative symptoms in schizophrenic patients (9,10). In contrast, subcortical (e.g., mesolimbic) dopaminergic hyperactivity has been suggested to mediate positive (psychotic) symptoms, such as delusions and hallucinations, in schizophrenia patients (11–13). These perceptual alterations in schizophrenia patients are sensitive to treatment with classical and atypical antipsychotic drugs. Blockade by these drugs of an overactive mesolimbic dopaminergic transmission may normalize an aberrant reward prediction induced by excessive dopaminergic transmission (14).

However, psychotic symptoms can be also evoked by nondopaminergic psychotomimetic agents, such as the noncompetitive N-methyl-*D*-aspartate receptor (NMDA-R) antagonists (15–19). Also, serotonergic agents such as lysergic acid diathylamine and related compounds, which are agonists of 5-HT_{2A} receptors, can produce perceptual and psychic alterations (20). DOI (1-[2,5-dimethoxy-4-iodophenyl-2-aminopropane]) is a partial 5-$HT_{2A/2C}$ agonist that evokes long-lasting alterations in consciousness and perception (20). DOI acts by overstimulating 5-HT_{2A} receptors, because its behavioral, neurochemical, and electrophysiologic effects are blocked by the selective 5-HT_{2A} receptor antagonist M100907 (21–23). However, the precise manner by which DOI evokes perceptual and psychic effects is not fully understood.

Activation of 5-HT_{2A} receptors by 5-HT or agonists elicits several electrophysiologic actions on cortical neurons recorded in vitro, increasing excitatory synaptic inputs (24,25) and changes in membrane properties, including membrane depolarization and reduction of the after-hyperpolarization that follows spike bursts (26,27). In vivo, 5-HT_{2A} receptor activation by endogenous 5-HT moderately increases pyramidal discharge (28,29), whereas systemic DOI administration evokes a dramatic increase in the firing rate of a subpopulation of PFC pyramidal neurons (23). However, despite the increasing knowledge of the cellular actions of hallucinogens, there is a lack of information concerning their actions at the network level.

We recently reported that the noncompetitive NMDA-R antagonist phencyclidine (PCP) markedly disrupts the cortical synchrony in the low-frequency range (.3–4 Hz) in rat PFC, an effect reversed by the antipsychotic drugs haloperidol (HAL) and clozapine (CLZ) (30). Here we extend these studies by showing that 1) DOI alters cortical synchrony in PFC similarly to PCP and 2) that this effect is also reversed by antipsychotic drug administration. Likewise, given previous reports suggesting an action of hallucinogens on thalamocortical 5-HT_{2A} receptors (25), we examined the dependence of the DOI's effect on the integrity of thalamocortical afferents to PFC.

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Methods and Materials

Animals

Male albino Wistar rats (250–320 g, Iffa Credo, Lyon, France) were kept in a controlled environment (12-hour light-dark cycle, 22 ± 2°C) with food and water provided ad libitum. Animal care followed the European Union regulations (O.J. of E.C. L358/1 18/12/1986).

Drugs and Reagents

Clozapine and DOI were obtained from Sigma/RBI (Natick, Massachusetts); M100907 [R-(+)-alpha-(2,3-dimethoxyphenil)-1-[4-fluorophenylethyl]-4-piperidinemethanol] (Lilly code LY 368675) was from Eli Lilly (Indianapolis, Indiana), and haloperidol (HAL) (intramuscular preparation) was from Laboratorios Esteve (Barcelona, Spain). Doses are expressed as free bases.

Electrophysiologic Experiments

Two sets of experiments were carried out. In the first series, we examined the effects of DOI on pyramidal cell firing in control and thalamic-lesioned rats. A preliminary report was published (23). The stored data corresponding to the neurons included in that report were reanalyzed to examine the effect of DOI on 1) burst firing, 2) local field potentials (LFP), and 3) temporal association between pyramidal discharge and LFP. Subsequently, a second series of experiments was carried out to examine the effects of antipsychotic drugs and M100907 on DOI-induced changes on LFP.

Single-unit extracellular recordings of pyramidal neurons were performed as previously described (23) in chloral hydrate–anesthetized rats. Pyramidal neurons were recorded extracellularly with glass micropipettes filled with 2 mol/L NaCl. The signal was amplified with a Neurodata IR283 (Cygnus Technology, Delaware Water Gap, Pennsylvania), postamplified and filtered with a Cibertec amplifier (Madrid, Spain) and computed online using a DAT 1401plus interface system Spike2 software (CED, Cambridge, United Kingdom). In the first series of experiments, LFPs were obtained by digitally filtering the signal using a band-pass filter at .1–60 Hz; LFPs in the experiments assessing the effects of antipsychotic drugs were obtained by online band-pass filtering the signal from the recording electrode as described (30).

Descents were carried out at anteroposterior (AP) +3.2 to +3.4, lateral (L) −5 to −1.0, dorsoventral (DV) −1.1 to 4.8 below the brain surface. To identify layer V–VI pyramidal neurons, stimulating electrodes were placed in two mPFC-innervated midbrain nuclei, the ventral tegmental area and the dorsal raphe nucleus (coordinates: dorsal raphe, AP −7.8, L −3.1, DV −6.8 with an angle of 30°; ventral tegmental area, AP −6.0, L −.5, DV −8.2) and were stimulated at .15–2 mA, .2 msec square pulses, .9 Hz to evoke antidromic spikes in pyramidal neurons of the mPFC. All recorded units were identified by antidromic activation and collision extinction with spontaneously occurring spikes (31). Only one recording per rat was performed (unit and/or LFP). In some cases, recording electrodes were filled with Pontamine sky blue to verify the recording site. Brain sections were stained with neutral red, according to standard procedures.

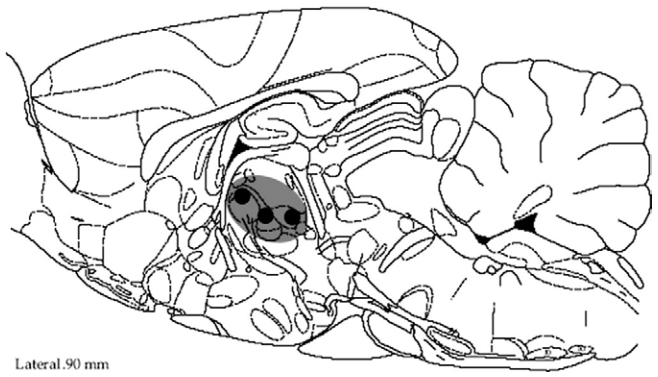

Figure 1. Sagittal view of the rat brain showing a schematic representation of the location of the tip of bipolar electrodes used to deliver electrolytic lesions in the thalamus. Stereotaxic coordinates for the three locations (black dots, from left to right) were as follows: 1) AP –1.8, L −.7, DV −5.7, 2) AP −2.7, L −.7, DV −6.3, and 3) AP −3.6, L −.7, DV −6.5, to target the mediodorsal and centromedial nuclei. The tip of electrodes was peeled off (~.5 mm), and poles were slightly separated (~.5 mm) to affect a larger tissue area. The shaded area shows the average extent of thalamic lesions. All rats showed extensive destruction of the centromedial and mediodorsal nuclei and also of the paracentral and paratenial nuclei. Additionally, most animals exhibited moderate to severe lesions of the anteromedial, centrolateral, parafascicular paraventricular, and reuniens nuclei, which also project to medial prefrontal cortex. Images of the actual thalamic lesions can be seen in Puig *et al.* (23). AP, anteroposterior; L, lateral; DV, dorsoventral.

Table 1. Increase in Activity of Pyramidal Neurons Produced by Systemic DOI Administration

	Basal	DOI
Firing rate (spikes/sec)	1.37 ± .48	5.80 ± .86[b]
% of spikes fired in bursts	52 ± 4	60 ± 4
Spikes in burst in 2 min	94 ± 39	460 ± 93[b]
Mean number of spikes per burst	2.13 ± .06	2.37 ± .08[a]
Number of bursts in 2 min	39 ± 15	181 ± 31[b]
Burst duration (msec)	14.43 ± 2.70	27.53 ± 2.99[b]
Interspike interval in bursts (msec)	11.92 ± 1.26	19.47 ± 1.19[b]
n	18	18

Data are means ± SEM.
[a] $p < .0005$.
[b] $p < .00001$.

Thalamic Lesions and Histological Examinations

Electrolytic lesions of the several thalamic nuclei projecting to the mPFC (32,33) were performed by passing 1.5-mA (2 pulses of 10 sec each; Grass Technologies (Quincy, Massachusetts) stimulation unit S48 connected to a Grass SIU 5 stimulus isolation unit) at three localizations: 1) AP −1.8, L −.7, DV −5.7, 2) AP −2.7, L −.7, DV −6.3, and 3) AP −3.6, L −.7, DV −6.5 (Figure 1). The

Table 2. Reduction of the Activity of Pyramidal Neurons Produced by Systemic DOI Administration

	Basal	DOI
Firing rate (spikes/s)	1.08 ± .32	.24 ± .07[a]
% of spikes fired in bursts	46 ± 3	23 ± 7
Spikes in burst in 2 min	65 ± 24	8 ± 3[a]
Mean number of spikes per burst	2.16 ± .06	2.03 ± 0.02 ($n = 8$)
Number of bursts in 2 min	30 ± 11	4 ± 2[b]
Burst duration (msec)	16.28 ± 3.07 ($n = 8$)	10.42 ± 1.36[a]
Interspike interval in bursts (msec)	13.68 ± 2.08 ($n = 8$)	10.14 ± 1.26[a]
n	12	12

Data are means ± SEM.
[a] $p < .05$.
[b] $p < .0005$.