



The natural hallucinogen 5-MeO-DMT, component of *Ayahuasca*, disrupts cortical function in rats: reversal by antipsychotic drugs

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Abstract

5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT) is a natural hallucinogen component of *Ayahuasca*, an Amazonian beverage traditionally used for ritual, religious and healing purposes that is being increasingly used for recreational purposes in US and Europe. 5-MeO-DMT is of potential interest for schizophrenia research owing to its hallucinogenic properties. Two other psychotomimetic agents, phencyclidine and 2,5-dimethoxy-4-iodo-phenylisopropylamine (DOI), markedly disrupt neuronal activity and reduce the power of low frequency cortical oscillations (<4 Hz, LFCO) in rodent medial prefrontal cortex (mPFC). Here we examined the effect of 5-MeO-DMT on cortical function and its potential reversal by antipsychotic drugs. Moreover, regional brain activity was assessed by blood-oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI). 5-MeO-DMT disrupted mPFC activity, increasing and decreasing the discharge of 51 and 35% of the recorded pyramidal neurons, and reducing (–31%) the power of LFCO. The latter effect depended on 5-HT_{1A} and 5-HT_{2A} receptor activation and was reversed by haloperidol, clozapine, risperidone, and the mGlu_{2/3} agonist LY379268. Likewise, 5-MeO-DMT decreased BOLD responses in visual cortex (V1) and mPFC. The disruption of cortical activity induced by 5-MeO-DMT resembles that produced by phencyclidine and DOI. This, together with the reversal by antipsychotic drugs, suggests that the observed cortical alterations are related to the psychotomimetic action of 5-MeO-DMT. Overall, the present model may help to understand the neurobiological basis of hallucinations and to identify new targets in antipsychotic drug development.

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Introduction

5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT) is a psychoactive compound found in *Ayahuasca*, an hallucinogenic beverage used in ritual ceremonies and for healing purposes (McKenna et al., 1984; Schultes and Hofmann, 1991; McKenna, 2004). *Ayahuasca* is being investigated for its potential clinical uses (McKenna, 2004). Its psychedelic effects include visual and auditory stimulation, mixing of sensory modalities, and deep psychological introspection. In addition to the methylated

indolamines, *Ayahuasca* contains β -carbolines, reversible inhibitors of monoamine oxidase-A (MAO-A), which prevent indoleamine deamination and increase their blood concentration and their psychedelic action (Aguirell et al., 1968; McKenna et al., 1984).

Several 5-HT_{2A} receptor (5-HT_{2A}-R) agonists, including 5-MeO-DMT, lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-iodo-phenylisopropylamine (DOI), mescaline and psilocybin possess hallucinogenic properties, altering perception, emotion and mood (Glennon, 1991, 1994; Nichols, 2004). The interest of psychedelic agents lies in their capacity to model certain aspects of psychosis in experimental research (Geyer and Vollenweider, 2008), helping also to identify brain areas/circuits altered in psychiatric disorders (Vollenweider et al., 1997a, b). Further, some of them may be useful in the treatment of psychiatric disorders (Vollenweider and Komater, 2010).

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Interestingly, not all 5-HT_{2A}-R agonists are hallucinogens, raising questions about the neural mechanisms responsible for their properties. Thus, differences in signaling pathways have been suggested (Kurrasch-Orbaugh et al., 2003; Gonzalez-Maeso et al., 2007).

5-MeO-DMT is synthesized and distributed for recreational purposes (Yu, 2008) and intoxications have been reported (Brush et al., 2004; Sklerov et al., 2005). Early reports suggested 5-MeO-DMT as a possible endogenous psychotoxin and several studies indicated its potential involvement in schizophrenia (Benington et al., 1965; Angrist et al., 1976; Gillin and Wyatt, 1976). In 2010 the Deputy Administrator of the Drug Enforcement Administration (DEA) placed 5-MeO-DMT into schedule I of the Controlled Substances Act (Drug Enforcement Administration (DEA), 2010).

The non-competitive N-methyl-D-aspartate receptor (NMDA) receptor antagonist phencyclidine (PCP) (Kargieman et al., 2007, 2012) and the preferential 5-HT_{2A}-R agonist DOI (Celada et al., 2008), markedly disrupt cortical function in rodents, increasing pyramidal neuron discharge and reducing low frequency cortical oscillations (LFCO) in medial prefrontal cortex (mPFC) (see (Celada et al., 2013) for review). Here we examined the effects of 5-MeO-DMT on cortical function in rats, and the potential reversal of these actions by antipsychotic drugs. Likewise, 5-MeO-DMT effects on regional brain activity were examined using functional magnetic resonance imaging (fMRI). The main objective of the study was to gain further insight into the neurobiological basis of hallucinations, helping also to identify new targets in schizophrenia treatment.

Materials and methods

Animals

Male albino Wistar rats (250–320 g) were used (Iffa Credo, France). Animal care followed the European Union regulations (O.J. of E.C. L358/1 18/12/1986) and was approved by the Institutional Animal Care and Use Committee.

Drugs and treatments

5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT), WAY-100635, clozapine (CLZ), risperidone (RIS) and clorgyline hydrochloride (CLG) were from Sigma/RBI (USA). M100907 [R-(+)-alpha-(2,3-dimethoxyphenyl)-1-[4-fluorophenylethyl]-4-piperidinemethanol] was a gift of Pierre Fabre Médicament (France), haloperidol (HAL) was from Laboratorios Esteve (Spain) and LY-379268 {(–)-2-oxa-4-aminobicyclo-[3.1.0]hexane-4,6-dicarboxylate} was from Tocris (UK).

All experiments were done in chloral hydrate anesthetized rats (400 mg/kg i.p. followed by 50–70 mg/kg/h using a perfusion pump). Drugs were administered intravenously (i.v) at the doses stated. To mimic *Ayahuasca* effects, preventing a rapid peripheral deamination of

5-MeO-DMT by MAO-A in lungs and liver, rats were pre-treated with the selective MAO-A inhibitor clorgyline (0.3 mg/kg) 15 min prior 5-MeO-DMT administration (Halberstadt et al., 2008). Seven groups of rats were administered with 5-MeO-DMT (0.1 mg/kg in all instances) followed by (a) saline, (b) CLZ (1 mg/kg), (c) HAL (0.1–0.2 mg/kg), (d) RIS (0.2 mg/kg), (e) WAY100635 (50–100 µg/kg), (f) M100907 (0.3 mg/kg), and (g) LY-379268 (0.5–2 mg/kg). Time between injections was 5 min.

To assess the effect of 5-HT_{1A}-R antagonist WAY100635, 5-HT_{2A}-R antagonist M100907 and mGluR2/3 agonist LY-379268 on LFCO we administrated intravenously cumulative doses (25–100 µg/kg), (0.15–0.6 mg/kg) and (0.5–2 mg/kg) of WAY100635, M100907 and LY-379268, respectively.

Electrophysiology: single unit and LFP recordings

Electrophysiological procedures were performed essentially as described elsewhere (Kargieman et al., 2007). Recordings of pyramidal neurons and oscillatory activity local field potential (LFP) were carried out in the mPFC (AP+3.2 to +3.4, L–0.2 to –0.5, DV –1.0 to –4; coordinates in mm (Paxinos and Watson, 2005)). All recorded pyramidal neurons were identified by antidromic activation from ventral tegmental area and collision test (Fuller and Schlag, 1976). In some experiments simultaneous recordings of oscillatory activity in the primary visual area (V1, AP–7.5, L–3.5) were performed using epidural electrodes.

After recording stable baseline activity for 5 min, clorgyline was slowly administered (30–45 s). 5-MeO-DMT was injected 15 min after clorgyline administration, followed by antipsychotics or receptor ligands 5 min later. At the end of experiments, rats were killed by anesthetic overdose. Brain sections were stained with Neutral Red, according to standard procedures, to verify the recording and stimulation sites.

fMRI protocol

fMRI experiments were conducted on a 7.0T BioSpec 70/30 horizontal animal scanner (Bruker BioSpin, Germany), equipped with a 12-cm inner diameter actively shielded gradient system (400 mT/m). The receiver coil was a phased array surface coil for the rat brain. fMRI was achieved using blood oxygenation level-dependent (BOLD) technique. Anesthesia was as in electrophysiological experiments, and MRI acquisition started 20 min after CLG administration (0.3 mg/kg, i.v.). TurboRARE images covering the whole brain were continuously acquired during 50 min (20 min before and 30 min after 5-MeO-DMT administration). Control animals received CLG and saline and were equally scanned for 50 min. Scan parameters were: echo time (TE)=22 ms, repetition time (TR)=1600 ms, 118 repetitions, field of view (FOV)=25×25×20 mm, matrix size=64×64×20 pixels, resulting in a spatial resolution of 0.39 in 1 mm slice thickness.

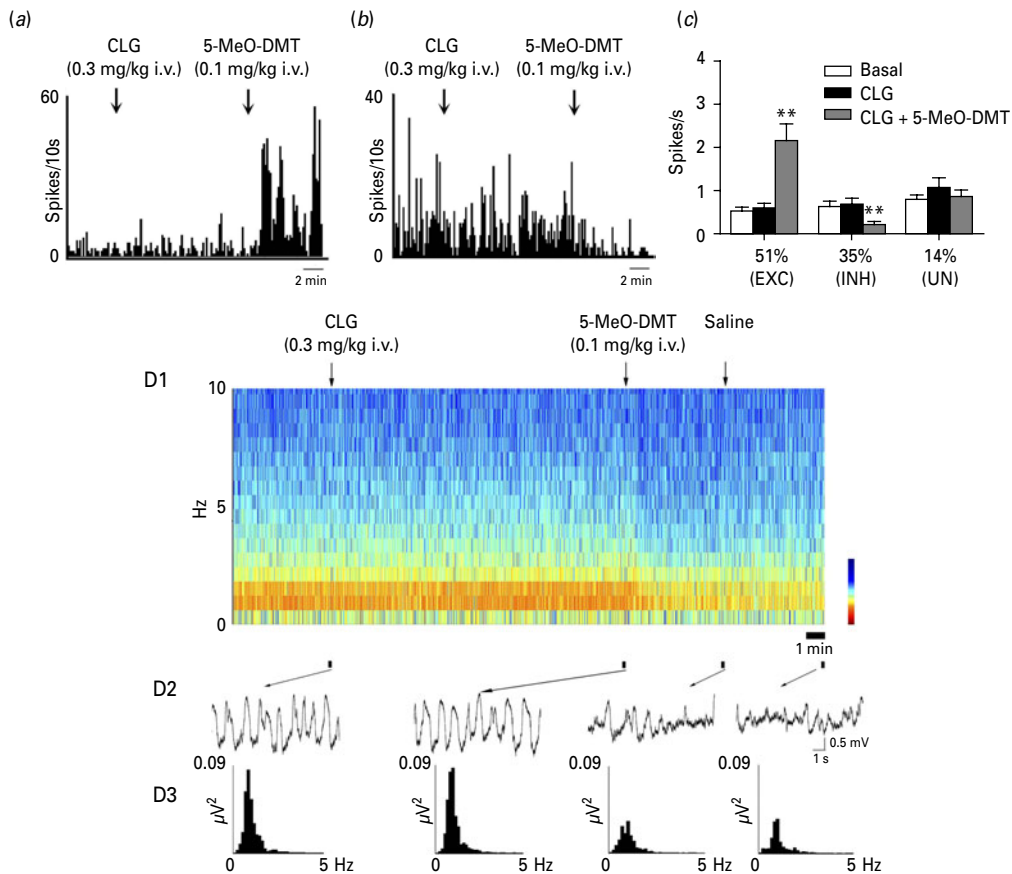


Fig. 1. Effect of 5-MeO-DMT (5-Methoxy-N,N-dimethyltryptamine) on pyramidal firing rate and low frequency cortical oscillation (LFCO) in rat medial prefrontal cortex (mPFC). (a and b) Examples of pyramidal neurons whose discharge was increased and decreased, respectively, by 5-MeO-DMT administration. (c) Bar graph showing the average effect of 5-MeO-DMT on firing rate according to the type of response. EXC, excited neurons ($n=19$); INH, inhibited neurons ($n=13$); UN, unaffected neurons ($n=5$). (D1) Spectrogram showing the effect of clorgyline and 5-MeO-DMT followed by saline on LFCO. Note the reduction in LFCO power, denoted by the color change, after 5-MeO-DMT administration. The intensity of the power spectrum is color-coded (red = high intensity; blue = low intensity). 5-MeO-DMT effect is long lasting (>10 min in this example). (D2) local field potential (LFP) recordings (10 s each) at the times indicated in the spectrogram. (D3) The power spectra of the above recordings (1 min each, around the above 10-s periods). Arrows mark drug injection. CLG: clorgyline hydrochloride.

To examine the effect of 5-MeO-DMT on arterial blood gas levels, the left femoral artery and vein were cannulated. Arterial blood samples (0.15 ml) were taken 5 min previous and 5, 10, 15 and 20 min after treatment (saline or 5-MeO-DMT) administration. CLG (0.3 mg/kg i.v.) was administered 20 min before arterial blood sample acquisition to mimic fMRI experimental conditions.

Data analysis

Firing rate was quantified by averaging the values in 2-min periods in each experimental period (4th–5th min after drug injection) and compared to pre-drug conditions (baseline or 5-MeO-DMT). Neurons were considered to be excited or inhibited when drugs induced a $\pm 30\%$ change of the discharge rate (Kargieman et al., 2007). Comparisons were made by determining the change for

each individual neuron and then coming up with the mean of those percentages changes. Burst analysis was carried out using previously described procedures (Laviolette et al., 2005). Off-line analysis was performed using the SPIKE 2 software (Cambridge Electronic Design, Cambridge, UK). Drug effects on LFCO (0.15–4 Hz) were analyzed, as follows. Power spectra were constructed (Fig. 1d) by using Fast Fourier Transformation (FFT) of 1-min signal intervals (band-pass filter of 0.1–100 Hz). Power data of 2-min periods were averaged, corresponding to baseline (4th–5th min), clorgyline (14th–15th min post-administration), 5-MeO-DMT (4th–5th min post-administration), and 5-MeO-DMT+drug (4th–5th min post-administration of last drug). Power resolution was 0.15 Hz. Results are given as area under curves (AUCs).

In MRI experiments, regions of interest (ROI; Fig. 2) were drawn over the first volume and signal intensity

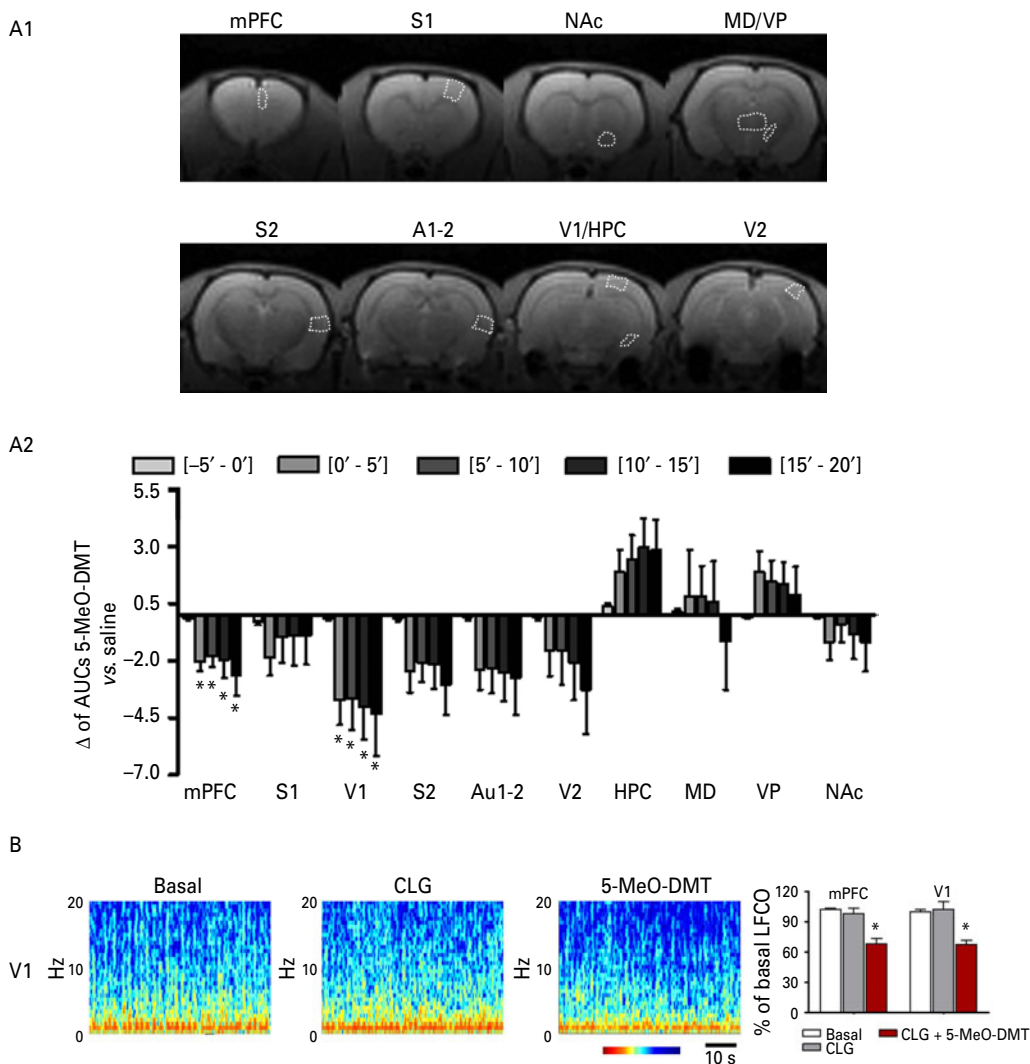


Fig. 2. Effect of 5-MeO-DMT (5-Methoxy-N,N-dimethyltryptamine) administration on blood-oxygen level dependent (BOLD) and low frequency cortical oscillation (LFCO) responses. (A1) Representative coronal slices showing the region of interest (ROI) analyzed. (A2) Bar graph showing the temporal effect of 5-MeO-DMT on BOLD signal in the different ROI: medial prefrontal cortex (mPFC), primary and secondary somatosensory cortex (S1 and S2, respectively), primary and secondary visual cortex (V1 and V2, respectively), auditory cortex (Au12), hippocampus (HPC), mediodorsal thalamus (MD), ventroposterior thalamus (VP) and nucleus accumbens (NAc). In order to show the effect of 5-MeO-DMT on functional magnetic resonance imaging (fMRI) signal intensity saline effect was subtracted from the effect of 5-MeO-DMT on the bar graph. $*p < 0.05$ vs. saline. (b) Spectrograms showing the effects of clorgyline and 5-MeO-DMT on LFCO in primary visual area (V1) and bar graph showing the averaged effects in mPFC and V1. $*p < 0.05$ vs. basal.

profiles were extracted for the next 117 volumes. After curve smoothing, AUCs of 5-min intervals were analyzed over a 25-min period corresponding to 5 min prior saline or 5-MeO-DMT administration and 20 min after saline or drug administration. Signal intensity changes after saline or 5-MeO-DMT were expressed as percentage of signal intensity change obtained in the 5 min period before the injection.

All data were analyzed using one- or two-way repeated-measures ANOVA (analysis of variance) followed by Newman-Keuls *post-hoc* test or paired Student's *t* test, as appropriate. Statistical significance was set at the 95% confidence level (two-tailed). Data are given as mean \pm S.E.M.

Results

Effects of clorgyline pre-treatment on mPFC activity

Clorgyline administration did not alter the firing rate of pyramidal neurons in mPFC (from 0.6 ± 0.1 to 0.7 ± 0.1 spike/s; n.s, paired Student's *t* test; $n = 30$, Fig. 1). Likewise, the power of LFCO remained stable (0.31 ± 0.02 vs. 0.31 ± 0.02 in basal and clorgyline periods; respectively; n.s paired Student's *t* test; $n = 60$).

Effects of 5-MeO-DMT on pyramidal neuron activity in mPFC

The effect of 5-MeO-DMT administration on pyramidal discharge was examined in 37 rats (one neuron per rat)

Table 1. Mean group weights and arterial blood gases measurements. Abbreviations: PaCO₂ – partial pressure of arterial CO₂; PaO₂ – partial pressure of arterial O₂; values were measured at the end of functional magnetic resonance imaging (fMRI) time series ([–5'–0'], [0'–5'], [5'–10'], [10'–15'], [15'–20'], respectively). Values expressed as mmHg and presented as mean±s.e.m.; n=4 and 5 for saline and 5-MeO-DMT (5-Methoxy-N,N-dimethyltryptamine) groups, respectively

Group	Weight (g)	paCO ₂ [0']	paCO ₂ [5']	paCO ₂ [10']	paCO ₂ [15']	paCO ₂ [20']
SALINE	304±20	46±2	46±1	45±1	44±1	44±1
5-MeO-DMT	305±15	40±3	42±2	43±2	41±1	41±1
Group	Weight (g)	paO ₂ [0']	paO ₂ [5']	paO ₂ [10']	paO ₂ [15']	paO ₂ [20']
SALINE	304±20	93±3	92±3	92±3	95±4	99±2
5-MeO-DMT	305±15	89±3	83±4	87±3	91±3	92±2

pretreated with clorgyline. When considering the individual change from baseline, 5-MeO-DMT increased the firing rate of 51% of the neurons (to 406% of baseline), reduced that of 35% (to 31% of baseline), and left the rest (14%) unaffected (Fig. 1c). Overall, 5-MeO-DMT increased pyramidal firing rate to 215% of baseline ($p < 0.001$, Student's t test; $n=37$). This was accompanied by an increase in the number of burst episodes (from 15 ± 2 to 37 ± 8 in 2-min periods, $p < 0.01$, Student's t test; $n=37$).

Given the experimental design (drugs administered 5 min after 5-MeO-DMT), we compared the effects of 5-MeO-DMT at 4th–5th and 9th–10th min post-administration, in order to avoid confounding drug effects with spontaneous reversals after 5-MeO-DMT. A group of rats was administered with saline 5 min after 5-MeO-DMT. One-way repeated-measures ANOVA of the firing rate data revealed a significant effect of the treatment [$F_{3,18}=10.2$, $p < 0.0004$, $n=7$] with significant *post-hoc* differences between 5-MeO-DMT and baseline and non-significant differences between the different times post-administration (0.5 ± 0.1 , 0.5 ± 0.2 , 1.7 ± 0.4 , 2.8 ± 0.7 spikes/s in basal conditions, after CLG, 5-MeO-DMT and saline, respectively).

Effect of 5-MeO-DMT on LFCO oscillations

In parallel with the effect on pyramidal discharge, 5-MeO-DMT significantly reduced the amplitude of LFCO in mPFC [$F_{2,114}=139.98$; $n=58$, $p < 0.000001$]. Power spectra were 0.31 ± 0.02 , 0.31 ± 0.02 and $0.20 \pm 0.01 \mu V^2$ during basal, CLG and 5-MeO-DMT periods, respectively. This effect was observed in all recordings, irrespectively of whether 5-MeO-DMT enhanced, reduced or left unaffected the discharge rate of the pyramidal neuron recorded simultaneously. Figure 1d shows a representative example of LFP recording. One-way repeated measures ANOVA revealed a significant effect of 5-MeO-DMT at min 4th–5th, 9th–10th and 14th–15th post-administration [$F_{4,12}=8.59$, $p < 0.002$, $n=4$] with significant *post-hoc* differences between 5-MeO-DMT and baseline and no significant differences between 4th–5th, 9th–10th and 14th–15th min after 5-MeO-DMT administration.

Effect of 5-MeO-DMT on BOLD signal

5-MeO-DMT administration significantly reduced the BOLD signal in mPFC and V1. Two-way ANOVA of the BOLD signal revealed a significant effect of the drug \times time interaction in mPFC [$F_{4,64}=3.39$; $p < 0.02$] and V1 [$F_{4,64}=3.41$; $p < 0.02$] $n=10$ and 8 for saline and 5-MeO-DMT, respectively, with significant *post-hoc* differences between 5-MeO-DMT and saline in all post-treatment periods (Fig. 2).

Since hallucinogenic drugs are known to have clear effects on the autonomic nervous system and blood pressure (McCall et al., 1987; McCall and Harris, 1988). In order to examine whether fMRI changes had such an origin, we assessed the effect of 5-MeO-DMT on arterial blood gas levels in the same experimental conditions and the same times acquisition than fMRI. A statistical comparison of the p_aCO₂ values using two-way ANOVA did not reveal any significant effect of treatment ([$F_{1,7}=3.07$, n.s.] nor treatment \times time interaction [$F_{4,28}=0.34$, n.s.], $n=4$ and 5 for saline and 5-MeO-DMT treatment. Similarly, two-way ANOVA revealed a non-significant effect of 5-MeO-DMT [$F_{1,7}=0.1925$; n.s.], and of the treatment \times time interaction [$F_{4,28}=1.09$, n.s.], $n=4$ and 5 for saline and 5-MeO-DMT on p_aO₂ values (Table 1).

Effect of 5-MeO-DMT on V1 oscillations

Given the change in BOLD signal in V1, we performed additional experiments to examine the effect of 5-MeO-DMT in this cortical area. Simultaneous recordings in mPFC and V1 indicated that 5-MeO-DMT administration concurrently reduced the amplitude of LFCO similarly in both cortical areas (Fig. 2). Two-way ANOVA revealed a significant effect of 5-MeO-DMT [$F_{2,12}=32.28$; $p < 0.0001$], for mPFC ($n=4$) and V1 ($n=4$), with no significant area differences nor treatment \times area interaction.

Antipsychotic drug reversal of 5-MeO-DMT-induced alterations on mPFC activity

Next, we examined whether clozapine and haloperidol could reverse the disruption of mPFC activity induced

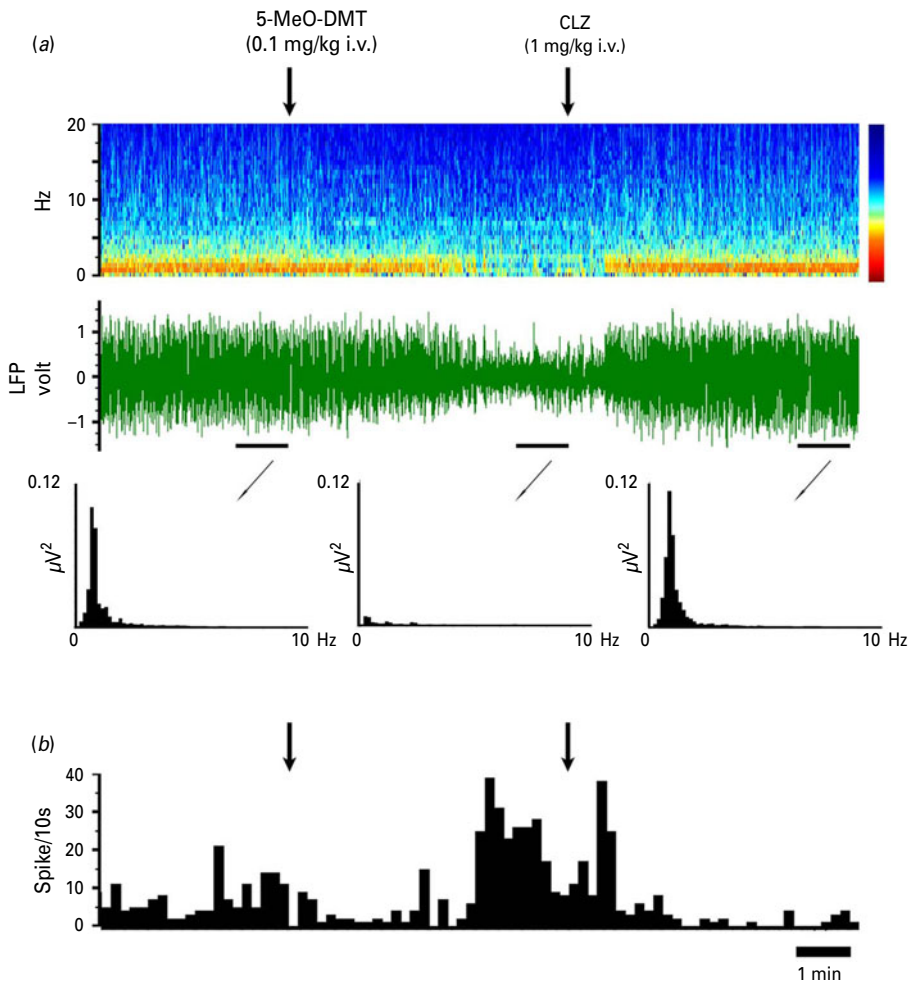


Fig. 3. Simultaneous single unit and low frequency cortical oscillation (LFCO) recordings showing the effect of 5-MeO-DMT (5-Methoxy-N,N-dimethyltryptamine) and its reversal by clozapine (CLZ). Note the simultaneous effect of the hallucinogen and its reversal by the antipsychotic on LFCO (a) and pyramidal discharge (b). Clorgyline was administered 15 min prior 5-MeO-DMT administration (not shown). LFP local field potential.

by 5-MeO-DMT. **Figure 3** shows an example of the concurrent effects of 5-MeO-DMT on pyramidal discharge and LFCO and the normalization of both variables induced by CLZ. One-way repeated-measures ANOVA revealed a significant effect of the treatment [$F_{3,15}=11.32$, $p<0.0005$, $n=6$]. *Post-hoc* analysis showed significant differences between baseline and 5-MeO-DMT periods and between 5-MeO-DMT and 5-MeO-DMT+CLZ periods.

Similarly, HAL administration reversed the increase in firing rate induced by 5-MeO-DMT. One-way repeated-measures ANOVA revealed a significant effect of the treatment [$F_{3,18}=4.39$, $p<0.02$, $n=7$]. *Post-hoc* analysis showed significant differences between baseline and 5-MeO-DMT periods and between 5-MeO-DMT and 5-MeO-DMT+HAL periods (**Fig. 4a-c**). Additionally, both antipsychotic drugs reversed the increase in burst episodes induced by 5-MeO-DMT (**Fig. 4d**).

In a subsequent set of experiments only involving LFCO recordings, we confirmed the ability of CLZ and

HAL to reverse 5-MeO-DMT effects on LFCO and examined whether risperidone (RIS) was also effective. The three antipsychotic drugs reversed the reduction in LFCO amplitude induced by 5-MeO-DMT. Two-way ANOVA of the LFCO data revealed a significant effect of post-treatment (saline or antipsychotic drug) [$F_{3,96}=100.16$; $p<0.00001$] and of group-by-post-treatment interaction [$F_{9,96}=9.24$, $p<0.0001$], $n=8, 11, 5, 12$ for saline, CLZ, RIS and HAL, respectively, with significant *post-hoc* differences between 5-MeO-DMT vs. 5-MeO-DMT+antipsychotic drug in all groups (**Fig. 4e, f**).

Involvement of 5-HT_{1A} and 5-HT_{2A} receptors in 5-MeO-DMT-induced effects

Given the *in vitro* affinity of 5-MeO-DMT for 5-HT_{1A}-R and 5-HT_{2A}-R receptors, we next examined which receptor(s) was(were) involved in the reduction of LFCO amplitude. The selective 5-HT_{2A}-R antagonist M100907 and the selective 5-HT_{1A}-R antagonist WAY100635

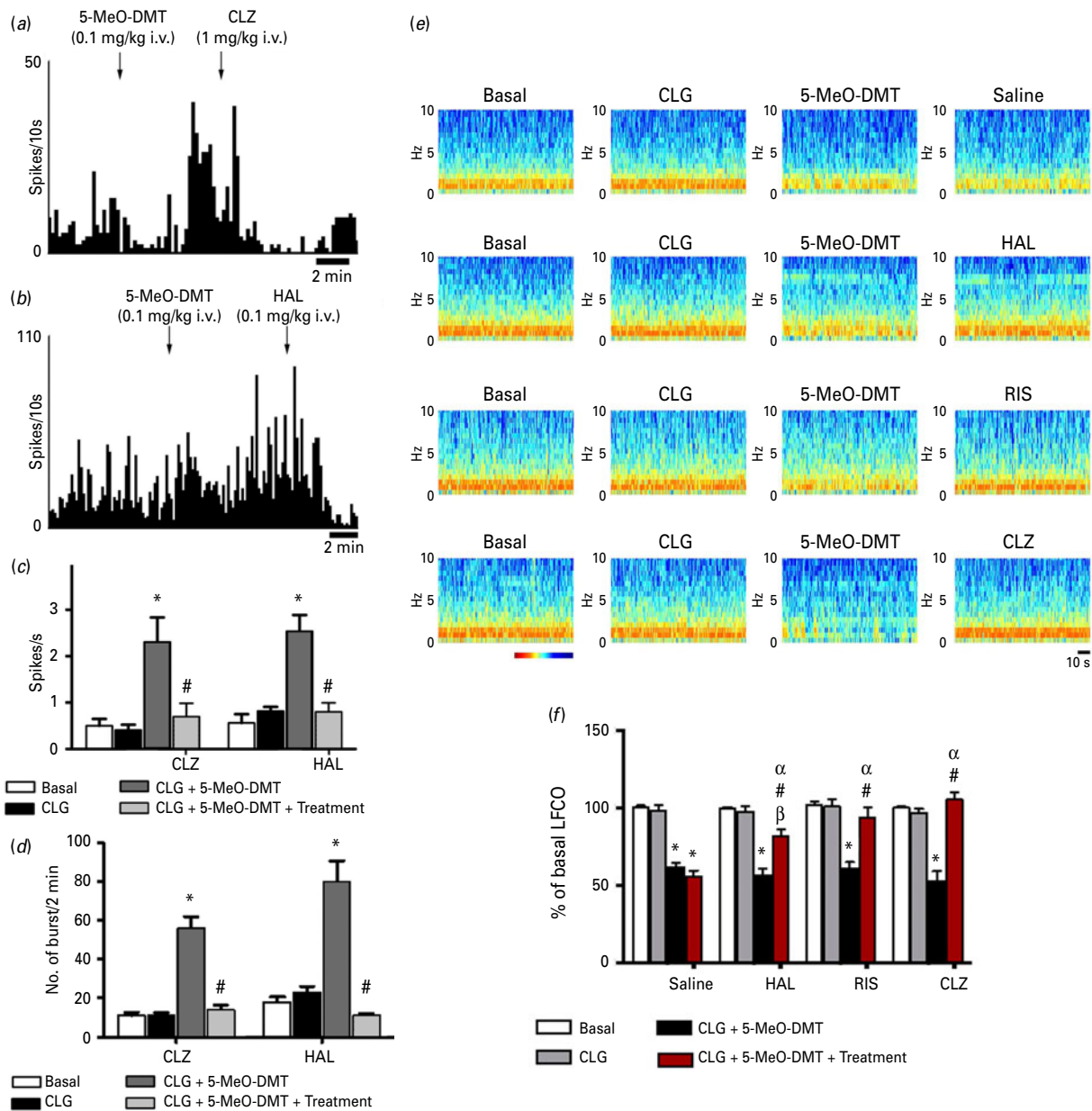


Fig. 4. Reversal by antipsychotic drugs of the effects of 5-MeO-DMT (5-Methoxy-N,N-dimethyltryptamine) on mPFC activity. (a and b). Examples of two pyramidal neurons showing an increase in the discharge rate after 5-MeO-DMT administration, which was reversed by the subsequent administration of clozapine (CLZ) (a) or haloperidol (b). (c) Bar graph showing the average effects of clorgyline; clorgyline+5-MeO-DMT and its reversal by clozapine ($n=6$) and haloperidol ($n=7$) on pyramidal discharge. (d) Bar graph showing the average effects of clorgyline; clorgyline+5-MeO-DMT and its reversal by clozapine ($n=6$) and haloperidol ($n=7$) on mPFC pyramidal neuron burst discharge. (e) Spectrograms showing the effects of the administration of saline, haloperidol (HAL), risperidone (RIS) and CLZ on 5-MeO-DMT-induced reduction on low frequency cortical oscillations (LFCO) (see doses in the text). (f) Bar graph showing the average effects on LFCO of saline, ($n=8$); HAL ($n=12$); RIS, ($n=5$); and CLZ, ($n=11$). * $p<0.01$ vs. basal; # $p<0.01$ vs. 5-MeO-DMT. $\alpha p<0.001$ vs. saline. CLG: clorgyline hydrochloride.

were administered after 5-MeO-DMT using the same treatment schedule than in previous experiments. Both agents reversed the fall in LFCO induced by 5-MeO-DMT in mPFC (Fig. 5). Two-way ANOVA analysis of the LFCO data revealed a significant effect of group-by-post-treatment interaction [$F_{6,39}=2.75$, $p<0.03$, $n=8, 4, 4$, for saline, M100907, and WAY100635 post-treatment, respectively], with significant *post-hoc*

differences between 5-MeO-DMT vs. baseline in all groups.

When administered alone neither antagonists altered the LFCO by itself (WAY100635: $102\pm 2\%$, $103\pm 7\%$, $107\pm 6\%$, $108\pm 6\%$, baseline-25-50-100 $\mu\text{g}/\text{kg}$ i.v, respectively; M100907: $100\pm 2\%$, $94\pm 11\%$, $83\pm 10\%$, $105\pm 10\%$, baseline-0.15-0.3-0.6 mg/kg i.v, respectively) (Fig. 6). One-way repeated-measures ANOVA of the LFCO revealed no

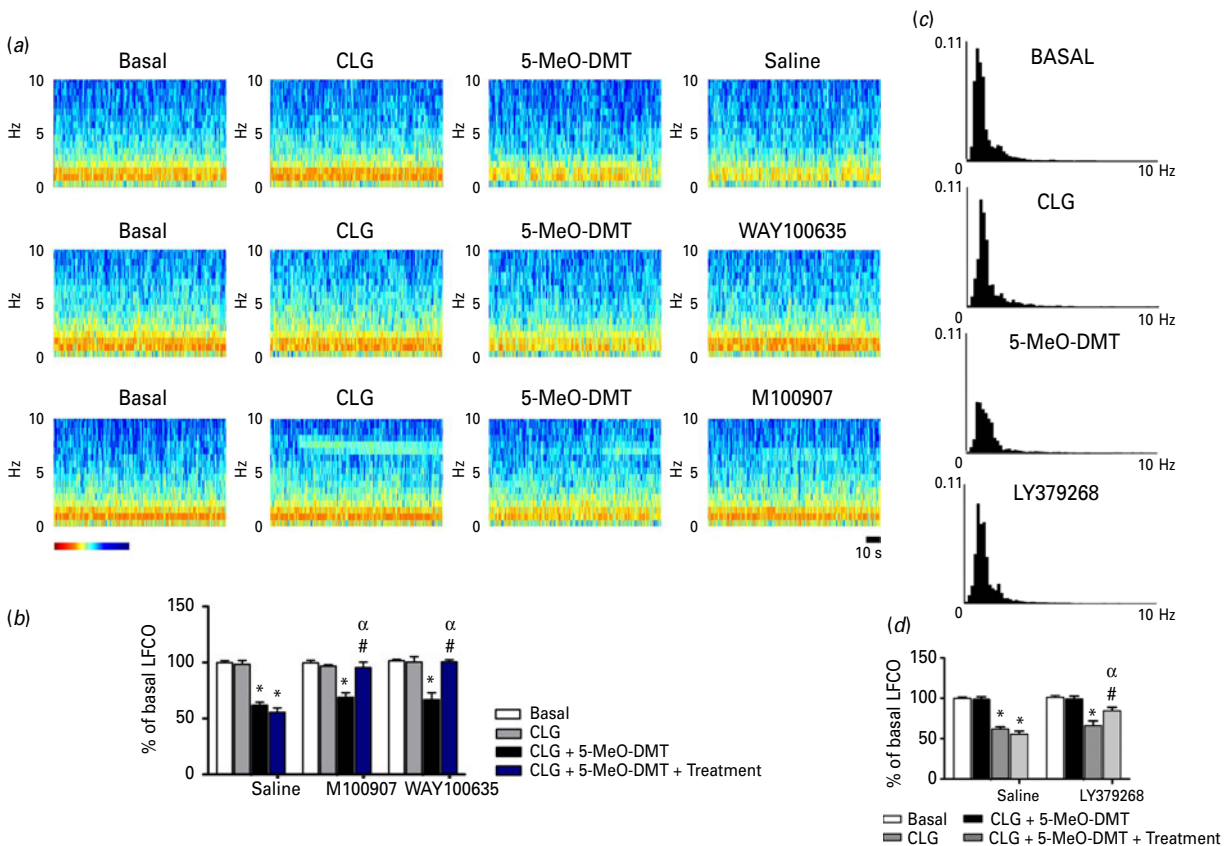


Fig. 5. Involvement of 5-HT_{1A} and 5-HT_{2A} receptors and of mGlu2/3R in the effect of 5-MeO-DMT (5-Methoxy-N, N-dimethyltryptamine) on low frequency cortical oscillation (LFCO). (a) Spectrograms showing the effects of saline, WAY100635 and M100907 on the 5-MeO-DMT-induced reduction of LFCO. (b) Bar graph showing the average effects of saline ($n=8$), WAY-100635 ($n=4$) and M100907, ($n=4$) on the reduction of LFCO induced by 5-MeO-DMT. (c) Power spectrum of a representative experiment showing the effect of 5-MeO-DMT and its reversal by LY-379268. (d) Bar graph showing the average effects of saline ($n=8$) and LY-379268 ($n=5$). * $p < 0.001$ vs. basal; # $p < 0.002$ vs. 5-MeO-DMT and $^{\alpha}p < 0.001$ vs. saline. CLG: clorgyline hydrochloride.

significant effect of 5-HT_{1A}-R antagonist WAY100635 (25–100 $\mu\text{g}/\text{kg}$ i.v.) treatment [$F_{3,9}=0.42$, n.s. $n=4$] nor 5-HT_{2A}-R antagonist M100907 (0.15–0.6 mg/kg i.v.) treatment [$F_{3,12}=1.73$, n.s. $n=5$].

Reversal of 5-MeO-DMT-induced alterations on LFCO by mGluR2/3 agonist

5-HT_{2A}-R and mGluR2/3 have been shown to form functional heterodimers that may be sensitive to the action of hallucinogens (Gonzalez-Maeso et al., 2008). We therefore examined whether the mGluR2/3 agonist LY-379268 could reverse the reduction in LFCO amplitude induced by 5-MeO-DMT. Two-way ANOVA analysis of the LFCO data revealed a significant effect of group-by-post-treatment interaction [$F_{3,33}=7.06$, $p < 0.001$, $n=8$ and 5 for saline, and LY-379268 post-treatment, respectively], with significant *post-hoc* differences between 5-MeO-DMT vs. baseline in all groups. Likewise, *post-hoc* test revealed a significant difference in the effect of saline and LY-379268 administration (Fig. 5c, d).

When administered alone the mGluR2/3 agonist LY-379268 induced a slight increase of LFCO ($104 \pm 2\%$,

$113 \pm 7\%$, $122 \pm 3\%$, $135 \pm 4\%$, baseline-0.5–1–2 mg/kg i.v., respectively). One-way repeated-measures ANOVA revealed a significant effect of the LY-3792668 (0.5–2 mg/kg i.v.) [$F_{3,9}=10.16$, $p < 0.003$, $n=4$] on LFCO. *Post-hoc* analysis showed significant differences between baseline and LY-379268 (1 mg/kg) period or LY-379268 (2 mg/kg) period (Fig. 6).

Discussion

The present study shows that 5-MeO-DMT, in conditions of MAO-A inhibition to mimic the effects of *Ayahuasca*, markedly disrupted cortical activity. Few previous studies examined the actions of 5-MeO-DMT on brain activity. 5-MeO-DMT and other serotonergic hallucinogens inhibited rat dorsal raphe cell firing (de Montigny and Aghajanian, 1977), whereas other studies reported electroencephalography (EEG) alterations in humans taking *Ayahuasca* (Riba et al., 2002, 2004). However, to our knowledge, no previous study examined the effects 5-MeO-DMT on cortical activity. Here we show that 5-MeO-DMT altered the discharge rate of 86% of the

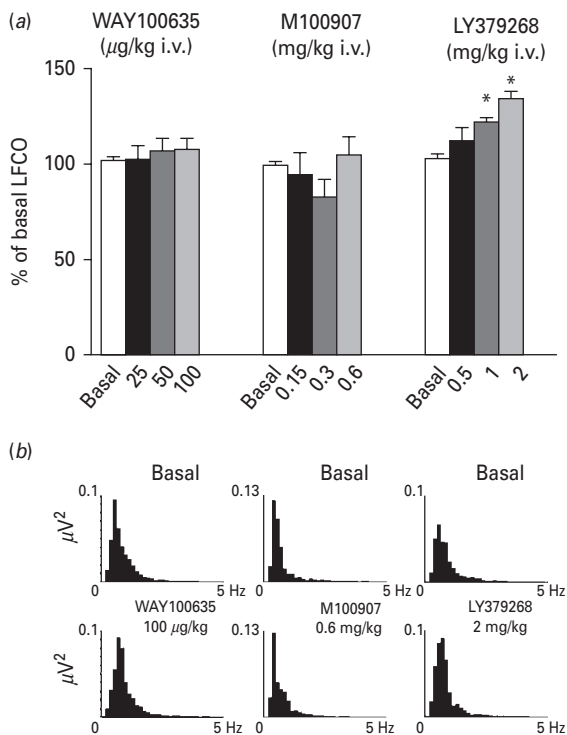


Fig. 6. Effect of 5-HT_{1A} receptor antagonist WAY100635, 5-HT_{2A} receptor antagonist M100907 and mGlu2/3 receptor agonist LY379268 on low frequency cortical oscillation (LFCO). (a) Bar graph showing the effect of cumulative doses of WAY100635, M100907 and LY379268 on LFCO. (b) Examples of power spectra of local field potential (LFP) recordings obtained in basal conditions and after WAY100635 (100 µg/kg), M100907 (0.6 mg/kg) and LY379268 (2 mg/kg) administrations **p* < 0.03 vs. basal.

recorded pyramidal neurons (51% excited, 35% inhibited) and increased the number of burst episodes. The opposite effects on neuronal discharge may result from the activation of 5-HT_{2A}-R in the recorded pyramidal neurons or in adjacent GABAergic interneurons, respectively, given the expression of 5-HT_{2A}-R in both neuronal types (Santana et al., 2004) and the involvement of GABAergic interneurons in the inhibitory actions of DOI (Puig et al., 2003; Wischhof and Koch, 2012).

In parallel to the individual changes in pyramidal neuron activity, 5-MeO-DMT reduced LFCO. Cortical oscillatory activity is a critical part of brain function due to its involvement in input selection, temporal coordination of activity, and synaptic plasticity (Buzsaki and Draguhn, 2004). Alterations in oscillatory activity have been associated with schizophrenia (Winterer and Weinberger, 2004; Uhlhaas and Singer, 2006; Ferrarelli et al., 2007) and the study of brain oscillations across frequencies has been proposed as a translational tool in schizophrenia research (Ford et al., 2007). Alterations in cortical oscillatory activity also occur in neurodevelopmental and pharmacological models of schizophrenia (Goto and Grace, 2006; Kargieman et al., 2007; Celada et al., 2008; Santana et al., 2011). The cortically generated slow

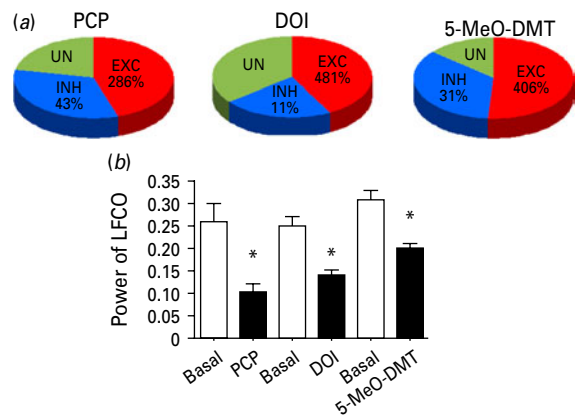


Fig. 7. Comparison of the effects of phencyclidine (PCP, 0.25 mg/kg i.v.), DOI (2,5-dimethoxy-4-iodo-phenylisopropylamine) (0.05–0.3 mg/kg i.v.) and 5-MeO-DMT (5-Methoxy-N, N-dimethyltryptamine) (0.1 mg/kg i.v.) on the discharge rate of pyramidal neurons (a) and on the power of low frequency cortical oscillation (LFCO) (b) % inside each sector is the average change vs. baseline in the discharge rate. EXC: excited neurons, INH: inhibited neurons and UN: unaffected neurons. Data from Kargieman et al., 2007; Puig et al., 2003; and Celada et al., 2008).

oscillation (LFCO, ~1 Hz) is a population variable that reflects the spontaneous changes of membrane potential in large neuronal ensembles during slow-wave sleep and anesthesia (from depolarized or 'up' states to hyperpolarized or 'down' states). LFCO groups other brain rhythms (Steriade, 2001, 2006), helping to establish temporal patterns of cortical activity and cortical-subcortical communication, as pyramidal neuron discharge occurs mainly during active (or 'up') phases of LFCO. In parallel with the effects on pyramidal discharge, 5-MeO-DMT markedly reduced LFCO in mPFC and V1, an action potentially related to its psychedelic activity. These alterations are similar to those produced by PCP and DOI (Kargieman et al., 2007, 2012; Celada et al., 2008; see Celada et al., 2013 for review). Hence, in common with these psychotomimetic agents (Fig. 7), 5-MeO-DMT evoked a disrupted activity state characterized by (1) altered pyramidal neuron discharge/pattern, and (2) reduced intensity of LFCO. However, the effect size was different for the three agents, being more marked for PCP (Kargieman et al., 2007), whereas DOI and 5-MeO-DMT evoked a smaller reduction (Celada et al., 2008). These differences may be related to the distinct psychotropic effects of PCP, DOI and 5-MeO-DMT.

5-MeO-DMT in conditions of MAO-A inhibition evokes behavioral alterations in rodents that may be related to its psychedelic activity in humans (Halberstadt et al., 2008; Halberstadt et al., 2012). Despite the present observations have been obtained in anesthetized rats they may also be related to these properties. Given the extensive projections of mPFC to many brain areas and the top-down PFC control of their activity (Groenewegen and

Uylings, 2000; Miller and Cohen, 2001; Gabbott et al., 2005), the 5-MeO-DMT-evoked alterations in PFC activity likely result in secondary activity changes in several brain networks. Hence, pyramidal neurons expressing 5-HT_{2A}-R project to midbrain monoaminergic nuclei (Jakab and Goldman-Rakic, 1998; Vazquez-Borsetti et al., 2009) which suggests downstream changes in monoaminergic activity. Additionally, 5-MeO-DMT-evoked changes in V1 can contribute to the hallucinogenic properties of *Ayahuasca* by altering nerve transmission in this area and creating 'false' visual impressions which would be subsequently processed by higher association cortical areas, such as the PFC, whose function is also altered by 5-MeO-DMT.

The present data are useful to understand the neurobiological basis of psychedelic action and suggest that alterations in primary sensory areas (V1) and association cortex (PFC) are involved. The relationship of the present observations with schizophrenia symptoms is however, less clear. A potential use of 5-MeO-DMT -in association with CLG- as psychotomimetic agent is supported by the similar changes evoked by 5-MeO-DMT and PCP (Kargieman et al., 2007) and by the reversal of its actions by marketed (CLZ, HAL, RIS) and potential (mGluR2/3 agonist) antipsychotic drugs. However, a main limitation is the fact that 5-MeO-DMT altered the function of V1 whereas schizophrenic patients show essentially auditory hallucinations. However, dysfunction across sensory systems and brain regions has been reported in schizophrenic patients. Hence, early neuronal encoding of visual stimuli in V1 is reduced in patients with schizophrenia (Seymour et al., 2013). Moreover, impaired efferent function in schizophrenia patients has been reported in the auditory (Ford and Mathalon, 2012; Ford et al., 2013) and visual systems (Spering et al., 2013), probably contributing to the pathophysiology of the hallucinations. Thus, 5-MeO-DMT, as well as other 5-HT_{2A} hallucinogens may be useful to elucidate brain areas/networks involved in certain schizophrenia symptoms but are unlikely to model the wide spectrum of psychotic symptoms.

The above electrophysiological observations were paralleled by changes in BOLD signal in mPFC and V1. The reduction in BOLD signal appears paradoxical, given the overall increase in discharge produced by 5-MeO-DMT and the relationship between neuronal discharge, energy consumption and blood flow. Early studies supported an association between spiking activity and BOLD signal (Boynton et al., 1996; Rees et al., 2000; Devor et al., 2003, 2005). However, other studies suggest a better correlation with oscillatory than with spiking activity (Logothetis, 2003; Shmuel et al., 2006; Viswanathan and Freeman, 2007). Interestingly, a recent study in human volunteers found negative BOLD signals in response to the psychedelic 5-HT_{2A}-R agonist psilocybin, in agreement with the present observations (Carhart-Harris et al., 2012). Yet, despite these similarities, further work is required

to understand the relationship between BOLD signal and neuronal activity.

5-MeO-DMT effects on LFCO were reversed by the selective antagonists WAY-100635 and M100907, which agrees with its *in vitro* affinity for 5-HT_{1A}-R and 5-HT_{2A}-R (Sills et al., 1984; McKenna and Peroutka, 1989). This is also in agreement with behavioral studies showing the involvement of 5-HT_{1A}-R in the actions of 5-MeO-DMT (Winter et al., 1999, 2000; Krebs-Thomson et al., 2006; van den Buuse et al., 2011). However, the biphasic effect of 5-MeO-DMT (plus CLG, as in the present study) on locomotor activity was antagonized by 5-HT_{2A}-R- but not 5-HT_{1A}-R-blockade (Halberstadt et al., 2008), suggesting a distinct role of 5-HT_{1A}-R in the different experimental models used. The involvement of 5-HT_{2A}-R in the suppression of LFCO agrees with a previous study (Celada et al., 2008), whereas that of 5-HT_{1A}-R is still unclear. Thus, the preferential post-synaptic 5-HT_{1A}-R agonist F15599 did not alter LFCO (Llado-Pelfort et al., 2010), while 8-OH-DPAT had a biphasic effect by itself but reversed PCP effects (Lladó-Pelfort et al., unpublished observations).

As previously observed with PCP and DOI (Kargieman et al., 2007; Celada et al., 2008), the effects of 5-MeO-DMT on neuronal firing and LFCO were reversed by HAL and CLZ (and RIS), acting mainly via D2-R (HAL) and 5-HT_{2A}-R blockade (CLZ, RIS) at the doses used (Schotte et al., 1993). The reversal by CLZ and RIS may result from the displacement of 5-MeO-DMT from 5-HT_{2A}-R sites by the antipsychotics. However, HAL effect must necessarily be interpreted by changes of dopamine networks, given the negligible occupancy of 5-HT_{2A}-R at the dose used (Schotte et al., 1993). Hence, ventral tegmental area stimulation excited fast-spiking interneurons and inhibited pyramidal neurons in mPFC, thus altering the excitatory/inhibitory balance (Tseng et al., 2006). Likewise, dopamine D2-R stimulation inhibits excitatory currents in mPFC pyramidal neurons (Tseng and O'Donnell, 2007). Given the presence of dopamine D2-R in pyramidal and GABAergic neurons (Santana et al., 2009), HAL may normalize the altered excitatory/inhibitory balance in mPFC, although further work is required to clarify the exact mechanism.

Serotonergic and glutamatergic neurotransmission play a significant role in the pathophysiology and treatment of schizophrenia (Marek and Aghajanian, 1998; Marek et al., 2000; Miyamoto, et al., 2005; Moreno et al., 2009). mGluR2/3 are potential new targets in schizophrenia treatment (Patil et al., 2007) (although Eli Lilly recently discontinued the development of the pro-drug used in that clinical trial). A 5-HT_{2A}-R/mGluR2 heterodimers appear to be involved in psychosis and mediates the psychedelic actions of serotonergic hallucinogens (Gonzalez-Maeso et al., 2008; Moreno et al., 2011). Interestingly, the antipsychotic-like activity of LY379268 requires the expression of 5-HT_{2A}-R (Fribourg et al., 2011). The present results agree with this body of evidence,

since the mGluR2/3 agonist LY379268 fully reversed the effect of 5-MeO-DMT on LFCO, as observed for CLZ, RIS and HAL. However, this effect might also derive from functional interactions between both receptors, given the modulatory role of 5-HT_{2A} receptors. The reduction of excitatory inputs onto PFC pyramidal neurons by LY379268 (Puig et al., 2003) may have altered 5-HT_{2A} receptor-mediated responses. However, despite pre-synaptic 5-HT_{2A} receptors have been postulated to mediate 5-HT_{2A}-mGluR2/3 interactions (Marek et al., 2000), their absence in glutamatergic axons of PFC (Miner et al., 2003) suggests a post-synaptic location.

Together with previous findings, the present results indicate that reductions in LFCO in PFC are a common signature of psychotomimetics drugs. The reversal of these effects by antipsychotic drugs with different mechanisms of action suggests a clear association with their therapeutic activity, regardless of their initial receptor target (D2R; 5-HT_{2A}R; mGluR2/3). This study further supports the usefulness of the LFCO model in PFC to examine the neurobiological basis of psychotic symptoms (in particular, hallucinations) and offers new perspectives to find new targets in the treatment of hallucinations as well as in antipsychotic drug development.

Statement of Interest

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