The serotonergic hallucinogen 5-Methoxy-N,N-dimethyltryptamine disrupts cortical activity in a regionally-selective manner via 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors

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Abstract

5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT) is a natural hallucinogen, acting as a non-selective serotonin $5-HT_{1A}/5-HT_{2A}$-R agonist. Psychotomimetic agents such as the non-competitive NMDA-R antagonist phencyclidine and serotonergic hallucinogens (DOI and 5-MeO-DMT) disrupt cortical synchrony in the low frequency range (<4 Hz) in rat prefrontal cortex (PFC), an effect reversed by antipsychotic drugs. Here we extend these observations by examining the effect of 5-MeO-DMT on low frequency cortical oscillations (LFCO, <4 Hz) in PFC, visual (V1), somatosensory (S1) and auditory (Au1) cortices, as well as the dependence of these effects on $5-HT_{1A}$-R and $5-HT_{2A}$-R, using wild type (WT) and $5-HT_{2A}$-R knockout (KO2A) anesthetized mice. 5-MeO-DMT reduced LFCO in the PFC of WT and KO2A mice. The effect in KO2A mice was fully prevented by the $5-HT_{1A}$-R antagonist WAY-100635. Systemic and local 5-MeO-DMT reduced 5-HT release in PFC mainly via $5-HT_{1A}$-R. Moreover, 5-MeO-DMT reduced LFCO in S1, Au1 and V1 of WT mice and only in V1 of KO2A mice, suggesting the involvement of $5-HT_{1A}$-R activation in the 5-MeO-DMT-induced disruption of V1 activity. In addition, antipsychotic drugs reversed 5-MeO-DMT effects in WT mice. The present results suggest that the hallucinogen action of 5-MeO-DMT is mediated by simultaneous alterations of the activity of sensory (S1, Au1, V1) and associative (PFC) cortical areas, also supporting a role of $5-HT_{1A}$-R stimulation in V1 and PFC, in addition to the well-known action on $5-HT_{2A}$-R. Moreover, the reversal by antipsychotic drugs of 5-MeO-DMT effects adds to previous literature supporting the usefulness of the present model in antipsychotic drug development.

1. **Introduction**

The serotoninergic hallucinogens evoke profound changes in perception, thought, mood and cognition (Nichols, 2004). Chemically, these agents are divided in two main classes: a) *indoleamines* such as lysergic acid diethylamide (LSD), psilocin, psilocybin, *N,N*-dimethyltryptamine (DMT) and 5-Methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) which bind with high affinity to several 5-HT receptors (5-HT-R), namely 5-HT$_{1A}$-R 5-HT$_{2A}$-R and 5-HT$_{2C}$-R and, b) *phenylalkylamines* such as mescaline and 2,5-dimethoxy-4-iodoamphetamine (DOI) which are highly selective for 5-HT$_{2A}$-R and 5-HT$_{2C}$-R (McKenna and Peroutka, 1989). The interest in serotoninergic hallucinogens lies in their capacity to model some schizophrenia symptoms by inducing mental states that resemble psychoses, also helping to study brain areas/circuits altered in psychiatric disorders (Vollenweider *et al*, 1998). Moreover, some of these agents were marketed in the past (e.g. LSD) as a therapeutic aid in psychoanalysis, and there is a growing interest in their therapeutic use for the treatment of mood and anxiety disorders (Vollenweider and Kometer, 2010).

5-MeO-DMT is a natural hallucinogen found in a variety of plant preparations (e.g., *Virola snuffs*) used for religious and recreational purposes (Shen *et al*, 2010). 5-MeO-DMT is a potent fast-acting hallucinogen with short duration of action in humans, and induces various physiological and behavioral changes in animal models (Halberstadt and Geyer 2011). 5-MeO-DMT is currently controlled in the United States as a Schedule I hallucinogen by the Drug Enforcement Administration. Like other indoleamine hallucinogens, 5-MeO-DMT shows high affinity for 5-HT$_{1A}$-R and 5-HT$_{2A}$-R (Sills *et al*, 1984; McKenna and Peroutka, 1989) and both receptors participate in its behavioral effects actions (Krebs-Thomson *et al*, 2006; Halberstadt and Geyer 2011; Winter *et al*, 2000).

Preclinical and clinical evidence supports that the psychotomimetic action of classical hallucinogens is mainly mediated by their agonistic actions at cortical 5-HT$_{2A}$-R (Aghajanian and Marek, 1999; Beique *et al*, 2007; Gonzalez-Maeso *et al*, 2007; Nichols, 2004). Although this is the prevailing view, other findings indicate that 5-HT$_{1A}$-R also play an important role in the behavioral effects of indoleamine hallucinogens (Krebs-Thomson *et al*, 2006; Van den Buuse *et al*, 2011; Winter *et al*, 2000) as well as in the mechanism of action of antipsychotic drugs (Bortolozzi et al., 2010; Kargieman *et al*, 2012; Newman-Tancredi and Kleven 2011). However, the exact role of 5-HT$_{1A}$-R activation in the psychotomimetic actions of indoleamine hallucinogens remains unclear.

Cortical oscillations have a key role in brain function due to their involvement in input selection, synaptic plasticity, memory consolidation and information processing (Buzsaki and Draguhn, 2004). Alterations in oscillatory activity have been associated with psychiatric disorders such as schizophrenia (Uhlhaas and Singer, 2010) and have been found in healthy volunteers after the consumption of psychotomimetic agents (Muthukumaraswamy *et al*, 2013; Riba *et al*, 2002). Moreover, alterations in

cortical oscillatory activity have been reported in neurodevelopmental and pharmacological models of schizophrenia (Celada *et al*, 2008; Goto and Grace, 2006; Kargieman *et al*, 2007; Riga *et al*, 2014).

Hence, previous studies showed that psychotropic agents with different mechanism of action, such as the non-competitive NMDA receptor antagonist phencyclidine (PCP) (Kargieman *et al*, 2007; 2012), the preferential 5-HT$_{2A}$-R agonist DOI (Celada *et al*, 2008) and the non-selective 5-HT$_{1A/2A}$-R agonist 5-MeO-DMT (Riga *et al*, 2014), markedly disrupted the activity of rodent prefrontal cortex (PFC), increasing pyramidal neuron discharge and reducing low frequency cortical oscillations (LFCO, <4Hz). Classical and atypical antipsychotic drugs reversed these alterations in all cases.

Given the limited knowledge of the brain areas/networks involved in hallucinogen action, the aim of the present study was to assess the effects of 5-MeO-DMT on cortical activity in anaesthetized mice. We used a combination of genetic (5-HT$_{2A}$-R knockout mice) and pharmacological approaches to 1) examine the effect of 5-MeO-DMT on LFCO in PFC and primary sensory areas, and 2) examine the role of 5-HT$_{1A}$-R and 5-HT$_{2A}$-R in the reduction of LFCO evoked by 5-MeO-DMT in the various cortical areas examined.

2. Materials and Methods

2.1. Animals

We used 9-16 week-old male homozygous 5-HT$_{2A}$-R knockout mice (referred as KO2A) and wild-type (WT) mice of the same genetic background (C57/BL6). Generation of KO2A strain has been reported elsewhere (Fiorica-Howells *et al*, 2002). Animal care followed the European Union regulations (directive 2010/63 of 22/09/2010) and was approved by the Institutional Animal Care and Use Committee. Stereotaxic coordinates were taken from bregma and duramater according to the mouse brain atlas (Franklin and Paxinos, 2008).

2.2. Drugs

5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT), risperidone (RIS) and WAY-100635 maleate were from Sigma/RBI (Natick, MA). Haloperidol (HAL) was from Laboratorios Esteve (Barcelona, Spain). Citalopram hydrobromide was from Tocris (Bristol, UK). Doses are expressed as free bases. All drugs were dissolved in saline and injected subcutaneously (s.c.). For the assessment of local effects in mPFC, 5-MeO-DMT was dissolved in the artificial cerebrospinal fluid (aCSF) used to perfuse the microdialysis probes (see below).

2.3. Electrophysiology

Electrophysiological procedures were performed as described elsewhere (Kargieman *et al*, 2012). Mice were anesthetized with chloral hydrate (400 mg/kg i.p.). Chloral hydrate was subsequently administered

using a perfusion pump (50-70 mg/kg/h) to maintain a constant level of anesthesia. Recordings of oscillatory activity (local field potential, LFPs) were carried out in the medial PFC (mPFC; AP+2.2 to +2.4, ML-0.2 to -0.4, DV-1.0 to -2.5 below brain surface; coordinates in mm). In most experiments simultaneous recordings of oscillatory activity in the primary somatosensory (S1, AP+0.5, ML+3.0), primary auditory (Au1, AP-2.8, ML+4.2) or primary visual (V1, AP-3.6, ML+2.5) cortices were also performed using epidural electrodes (electrocorticograms, ECoGs). LFP and ECoGs signal were amplified (X 1000 and X2000 respectively) and filtered between 0.1-100 Hz.

After recording stable baseline activity for 5 min, drugs were administered. 5-MeO-DMT (1 mg/kg) was slowly injected followed by saline (in all genotypes) or the antipsychotics HAL (0.6 mg/kg) or RIS (1 mg/kg) in WT mice. Time between injections was 12 minutes. To further evaluate the role of the $5\text{-}HT_{1A}\text{-}R$ on 5-MeO-DMT induced disruption of prefrontal activity, KO2A mice were pretreated (5 minutes after recording stable baseline activity) with saline or the selective $5\text{-}HT_{1A}\text{-}R$ WAY-100635 (0.5 mg/Kg) before 5-MeO-DMT administration. WAY-100635 dose was chosen from the literature owing to its ability to antagonize behavioural effects of 5-MeO-DMT (Halberstandt *et al*, 2011).

2.4. Intracerebral microdialysis

Extracellular serotonin (5-HT) concentrations were measured by *in vivo* microdialysis as previously described (Amargós-Bosch *et al*, 2004). Briefly, one concentric dialysis probe (membrane 2 mm long) was implanted in mPFC (AP +2.2; ML −0.2; DV −3.4 from skull). Microdialysis experiments were carried out in freely moving mice 20-24 h after surgery. Probes were continuously perfused with aCSF (in mM: NaCl, 125; KCl, 2.5; CaCl2, 1.26 and MgCl2, 1.18) pumped at 1.5 μl/min and containing 1 μM citalopram to prevent 5-HT reuptake. In these conditions, the extracellular 5-HT concentration is representative of the spontaneous 5-HT release by nerve terminals (Adell *et al*, 2002). Dialysate samples were collected every 20 min.

After an initial 60 min stabilization period, four baseline samples were collected before systemic or local (intra-mPFC) pharmacological treatments. 5-HT concentrations was analysed by HPLC-amperometric detection (Hewlett Packard-1049, Palo Alto, CA, USA) at +0.60 V, with detection limit of 2 fmol/sample.

Moreover, side-to-side head weaving (head twitch response, HTR) was scored for 4 consecutive 5-min periods by direct observation of mice undergoing *in vivo* microdialysis, in basal conditions and after 5-MeO-DMT administration (González-Maeso *et al*, 2007).

At the end of experiments, mice were killed by anesthetic overdose. Brain sections were stained according to standard procedures, to verify recordings sites and proper probe placement.

2.5. Data and statistical analysis.

Off-line analysis of electrophysiology results was performed using the Spike2 software. Drug effects on LFCO were analyzed, as follows. For each condition (baseline, 5-MeO-DMT, WAY-100635 or saline + 5-MeO-

DMT and 5-MeO-DMT + antipsychotic or saline), the power spectrum of 3 minutes signal was analyzed off-line using Spike2 software built-in and self-developed routines. Eighteen consecutive ten-second periods were subjected to a Fast Fourier Transformation, for frequencies from 0.15 to 80 Hz, with a resolution of 0.15 Hz. For statistical analyses, the mean values of the LFCO power (0.15–4 Hz) were quantified. Data were expressed as percentage of baseline and are given as mean ± SEM.

Microdialysis data are expressed as fmol/30 µl for 5-HT and shown in the figures as percentages of basal values, averaged from four fractions collected before treatment. Normalized areas under curve values (AUCs) were also calculated to compare genotypes.

Stereotypes were rated during the last 20 min before drug administration and the first 20 min post-drug administration and were divided in four 5-min blocks. HTR was quantified as the number of occurrences during the observation period. Total scores for each animal were calculated by averaging the individual values during each 5-min period.

Results are given as (mean±SEM). All data were analyzed by Student's t-test or two-way repeated-measures analysis of variance (ANOVA), with treatment (or area) and genotype as factors, followed by Newman–Keuls post-hoc test, as appropriate. The level of significance was set at $p < 0.05$.

3. Results

3.1. *Characteristics of LFCO in mouse cortical areas*

As previously reported (Kargieman *et al*, 2012), the power spectra of LFCO in mouse mPFC did not differ between genotypes (WT: 0.054±0.004; KO2A: 0.064±0.004 μV^2; n.s Student's t-test; n=40 and 22, respectively). Similarly, there were no differences among genotypes in the power spectra of LFCO in S1 (WT: 0.053±0.007; KO2A: 0.087± 0.019 μV^2; n.s Student's t-test; n=11 and 10, respectively), Au1 (WT: 0.041±0.012; KO2A: 0.027±0.018 μV^2; n.s Student's t-test; n=11 and 10, respectively) and V1 (WT: 0.065± 0.011; KO2A: 0.073±0.023 μV^2; n.s Student's t-test; n=10 and 6, respectively).

3.2. *Effect of 5-MeO-DMT on LFCO in mPFC of WT and KO2A mice*

As reported in rats (Riga *et al*, 2014), systemic 5-MeO-DMT administration significantly reduced LFCO in the mPFC of WT mice. Interestingly, 5-MeO-DMT differently reduced LFCO in WT and KO2A mice (WT: from 0.054±0.004 to 0.030±0.002 μV^2 (51.1±2.5% of baseline), n=40; KO2A: from 0.064±0.004 to 0.041±0.004 μV^2 (61.4±3.3% of baseline), n=13). Two-way ANOVA revealed significant effects of 5-MeO-DMT ($F_{(1,51)}=120.21$; $p < 0.00001$) and genotype ($F_{(1,51)}=4.99$; $p < 0.03$) with no significant treatment x genotype interaction
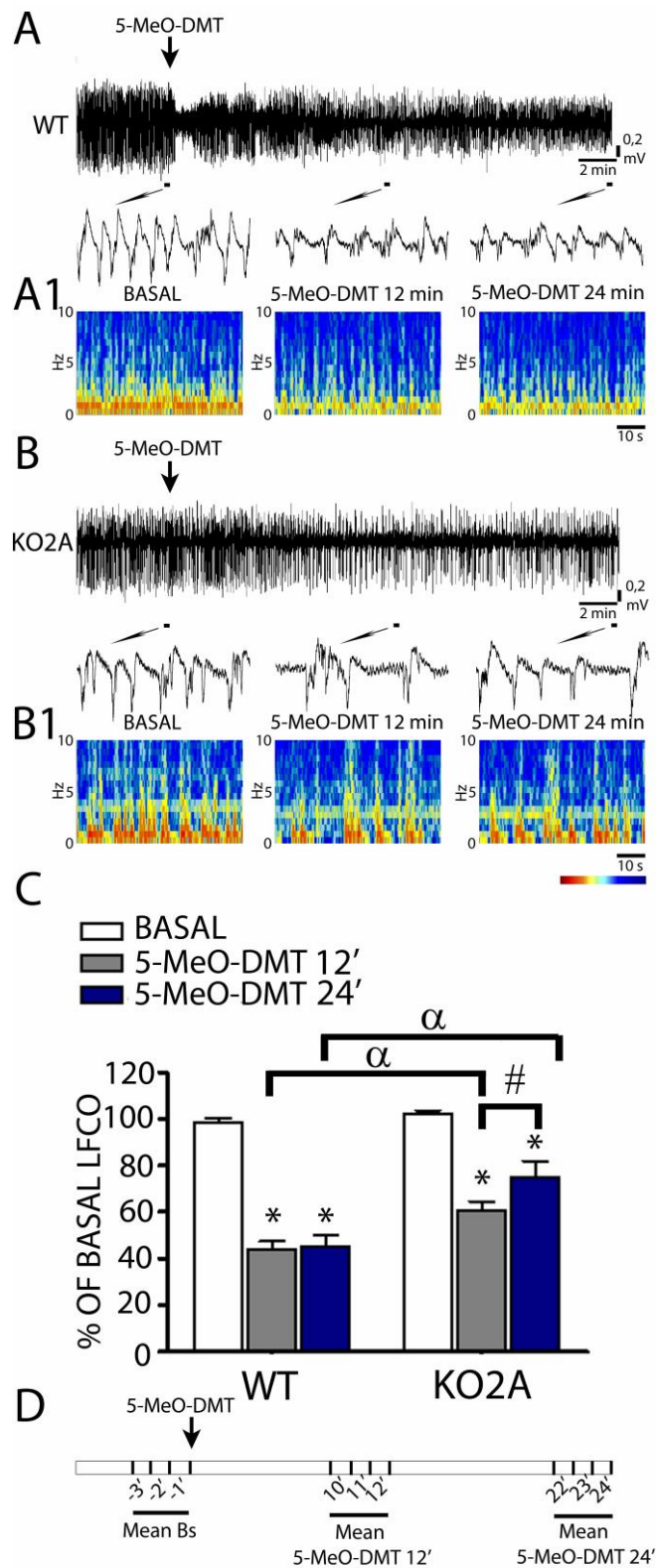
Figure 1. Effect of subcutaneous administration of 5-MeO-DMT on the low frequency cortical oscillations (LFCO) in mPFC of WT and KO2A mice. **A)** and **B)** Local field potential (LFP) recordings in WT **A)** and KO2A **B)** mice of representatives experiments showing de decrease in LFCO after 5-MeO-DMT administration. Small bars below the recording denote the 10-second period corresponding to the LFP shown below. **A1)** and **A2)**

Spectrograms showing the effect of the administration of 5-MeO-DMT in a 1-min period. Time bars in abscissa are 10 s; ordinates are in Hz. The intensity of the power spectrum is color-coded (red = high intensity; blue = low intensity). **C)** Bar graph showing the effects of 5-MeO-DMT on LFCO in WT and KO2A mice. **D)** Scheme showing the periods where LFP were quantified * $p < 0.0002$ vs baseline; [α] $p < 0.005$ 5-MeO-DMT (WT) *vs* 5-MeO-DMT (KO2A); [#] $p < 0.005$ *vs* 5-MeO-DMT (12 vs 24 min) in KO2A mice; n=13 and 7 for WT and KO2A mice, respectively.

To examine the kinetics of 5-MeO-DMT effect on LFCO in WT and KO2A mice, we measured the effect of subcutaneous administration of 5-MeO-DMT 12 and 24 min post-administration in WT and KO2A mice. 5-MeO-DMT differentially reduced LFCO in the mPFC of WT (to 44.1±3.3% and 45.4±4.7% of basal values 12 and 24 min after 5-MeO-DMT) and KO2A mice (to 60.5±4.0% and 75.2±6.8% of basal values 12 and 24 min after 5-MeO-DMT). Figures 1A and 1B show two representative examples of the effect of 5-MeO-DMT on LFCO in the two genotypes. Two-way ANOVA revealed significant effects of 5-MeO-DMT ($F_{(2,36)}=136.05$; $p < 0.00001$), genotype ($F_{(1,18)}=13.24$; $p < 0.002$) and treatment x genotype interaction ($F_{(2,36)}=8.83$; $p < 0.001$). Post-hoc analysis showed significant differences between baseline and 5-MeO-DMT and between 5-MeO-DMT effects at the two post administration times analyzed in the two genotypes (Figure 1C and 1D).

3.2.1. *Effect of 5-MeO-DMT in mPFC of KO2A mice: role of 5-HT$_{1A}$ receptors*

Given the differential effect of 5-MeO-DMT in WT and KO2A mice, we examined the potential involvement of 5-HT$_{1A}$-R. Pretreatment with the 5-HT$_{1A}$ receptor antagonist WAY-100635 (0.5 mg/kg s.c.) fully prevented the 5-MeO-DMT-evoked reduction of LFCO in the mPFC of KO2A mice (Figure 2). Interestingly, WAY-100635 increased the power of LFCO by itself. Two-way ANOVA revealed a significant effect of 5-MeO-DMT treatment ($F_{(2,34)}=14.29$, $p < 0.005$), WAY-100635 pre-treatment ($F_{(2,17)}=32.75$, $p < 0.0001$) and of treatment x pre-treatment interaction ($F_{(4,34)}=7.28$, $p < 0.0002$), with significant post-hoc differences between saline and WAY-100635 pre-treatments and between saline+5-MeO-DMT and WAY-100635+5-MeO-DMT treatments (Figure 2B).
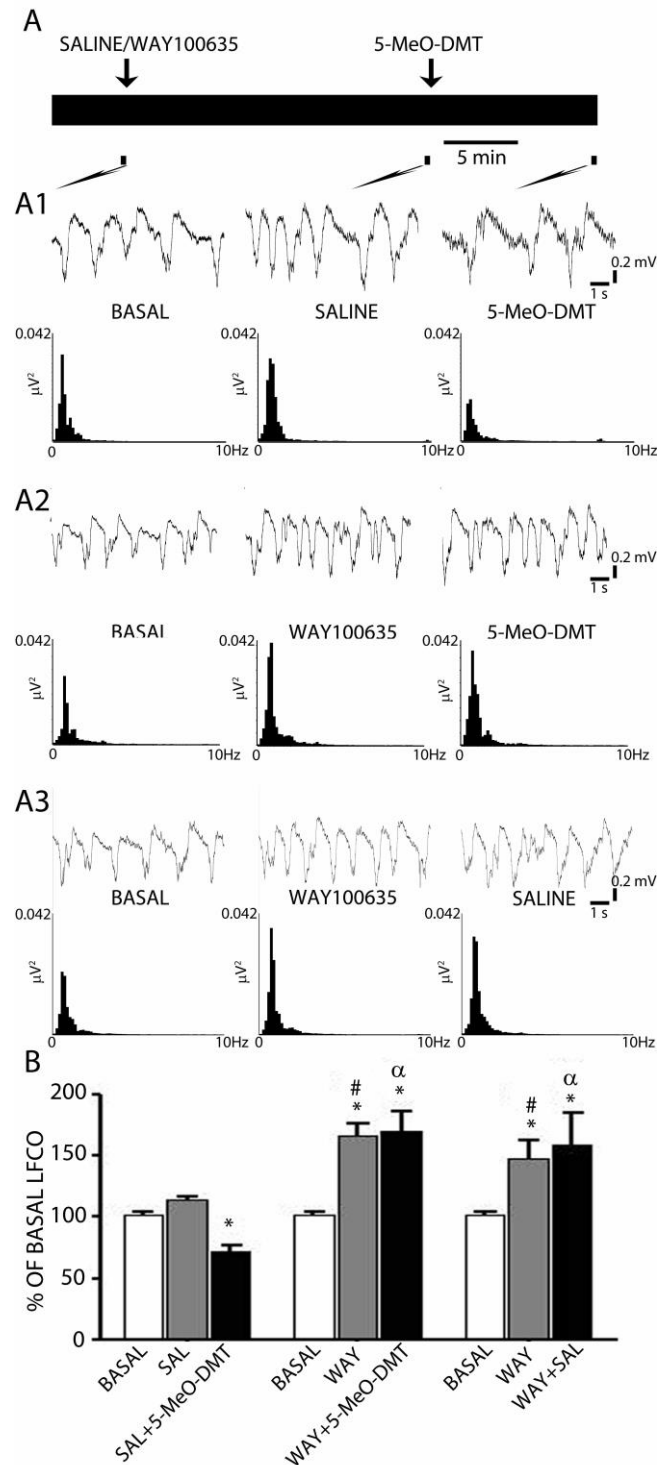
Figure 2. The 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) antagonist WAY-100635 prevents the 5-MeO-DMT effect on low frequency cortical oscillations (LFCO) in KO2A mice. **A)** Scheme of the protocol used and representative examples of local field potential (LFP) recordings and corresponding power spectrums in mPFC from KO2A mice treated with saline+5-MeO-DMT (1 mg/kg s.c) **A1)**, WAY-100635 (0.5 mg/kg s.c)+5-MeO-DMT **A2)** and WAY100635+saline **A3)** LFPs show the effect of the administration of saline or WAY-100635 plus 5-MeO-DMT or saline in the time periods (10 s) shown in the upper scheme. Power spectrums of 1-min period show a decrease of LFCO (0.15-4 Hz) after 5-MeO-DMT administration only in KO2A mice pretreated with

saline. Note that WAY-100635 by itself increases LFCO. **B)** Bar graph showing the prevention by WAY-100635 pretreatment of 5-MeO-DMT-evoked reduction on LFCO in KO2A mice. *p<0.05 *vs* basal; #p<0.03 *vs* saline; $^{\alpha}$p<0.0002 *vs* saline+5-MeO-DMT. n=6, 7 and 7 for saline+5-MeO-DMT, WAY100635+5-MeO-DMT and WAY100635+saline groups, respectively.

3.3. Effects of 5-MeO-DMT on 5-HT release in mPFC and behavioural scores

Basal extracellular concentrations of 5-HT in dialysed samples of mPFC were WT: 14.5±1.8 (n=15); KO2A: 16.2±2.3 (n=11) fmol/30 µl. Non-significant differences between genotypes were found in basal 5-HT concentrations.

The systemic administration of 5-MeO-DMT (1 mg/kg s.c) decreased extracellular 5-HT concentration comparably in the mPFC of WT and KO2A mice (Figure 3A). The maximal decreases were to 57.0±7.0% and 43.6±4.9% of baseline for WT and KO2A mice, respectively. Two-way ANOVA revealed a significant effect of 5-MeO-DMT ($F_{(9,99)}$=8.35; p<0.00001) with no significant effects of genotype and genotype x treatment interaction.

In parallel, 5-MeO-DMT produced a significant increase in the spontaneous HTR rate in WT but not in KO2A mice (from 0.98±0.29 to 4.09±0.66 in WT and from 0.92±0.34 to 0.39±0.15 in KO2A mice after 5-MeO-DMT administration) (Figure 3B). Two-way ANOVA showed a significant effect of 5-MeO-DMT ($F_{(1,11)}$=8.28; p<0.02), genotype ($F_{(1,11)}$=21.21; p<0.001) and genotype x treatment interaction ($F_{(1,11)}$=16.41; p<0.002).
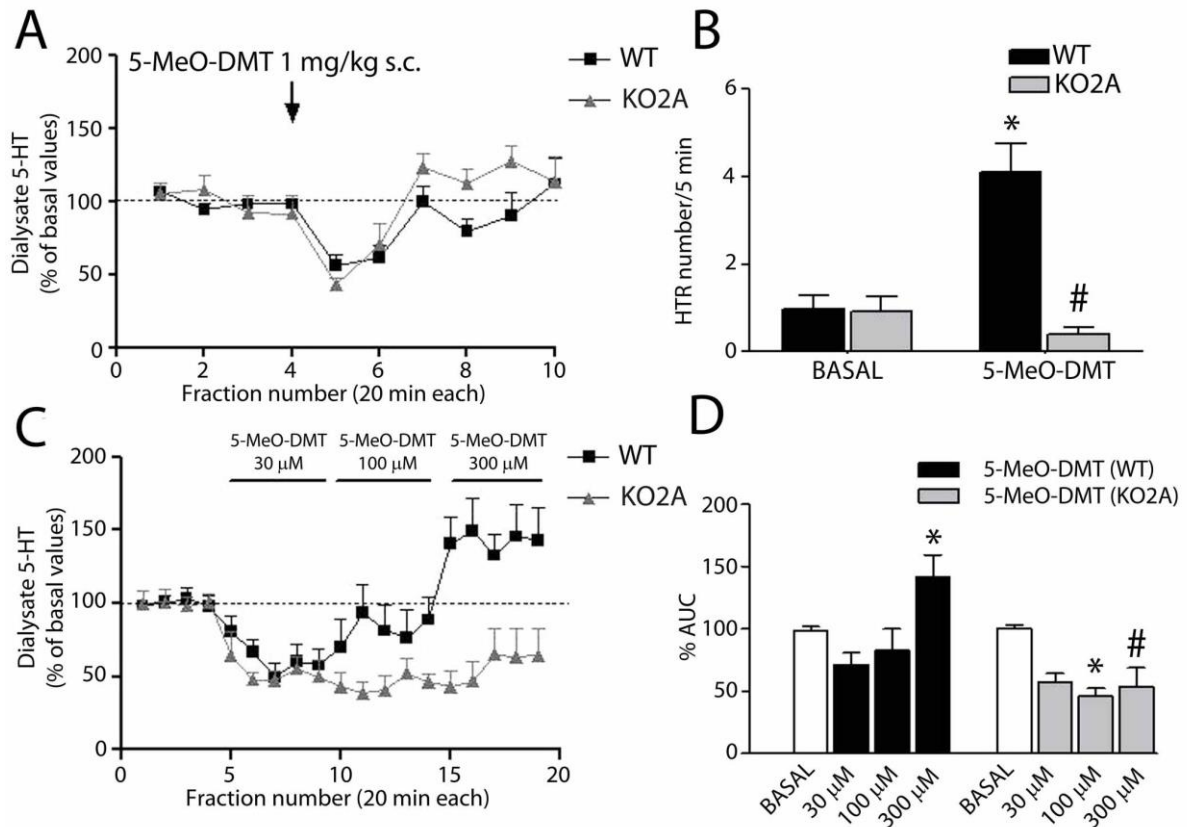
Figure 3. Effect of 5-MeO-DMT administration on the mPFC 5-HT extracellular concentration in freely-moving WT and KO2A mice and behavioural stereotypes mediating by serotonin 5-HT$_{2A}$ receptor activation (Head twitch response –HTR-). The subcutaneous (1 mg/kg) administration of 5-MeO-DMT **A)** decreases 5-HT extracellular concentration (n=7 and 6 for WT and KO2A mice, respectively) and **B)** increases HTR in WT (n=7) but not in KO2A mice (n=6). * p<0.001 *vs* basal; #p<0.001 5-MeO-DMT (WT) *vs* 5-MeO-DMT (KO2A). Note that effects are moderate but statistically significant. **C)** and **D)** The local application of 5-MeO-DMT (30-100-300 μM) by reverse dialysis in mPFC dose-dependently elevated mPFC 5-HT extracellular concentration in WT mice (n=8) and decrease mPFC 5-HT extracellular concentration in KO2A mice (n=5). Data are shown as % of basal values. * p<0.05 *vs* basal; $^{\#}$ p<0.0003 5-MeO-DMT 300 μM (WT *vs* KO2A).

3.3.1. *Effects of local 5-MeO-DMT administration on 5-HT release in mPFC*

5-HT$_{1A}$ autoreceptors play a major role in the control of the ascending serotonergic system. Likewise, there is an additional control of serotoninergic activity by postsynaptic 5-HT$_{1A}$-R via direct descending inputs from PFC to the raphe nuclei (Celada et al., 2001; Gabbott et al, 2005; Vazquez-Borsetti et al, 2009)". Therefore, in order to discriminate the involvement of presynaptic and postsynaptic 5-HT$_{1A}$-R in the reduction of 5-HT release induced by 5-MeO-DMT, we locally applied the compound in mPFC by reverse dialysis. The local perfusion of 5-MeO-DMT (30, 100, 300 μM) by reverse dialysis dose-dependently altered the 5-HT concentration differently in the mPFC of WT and KO2A mice (Figure 3C). Two-way ANOVA revealed

significant effects of 5-MeO-DMT (F(18,198)=5.11; p<0.00001), genotype (F(1,11)=9.27; p<0.02) and genotype x treatment interaction (F(18,198)=3.73; p<0.00001). The lower concentration used (nominal 30 µM) evoked a similar reduction of extracellular 5-HT in WT and KO2A mice. However, higher concentrations clearly discriminated between WT and KO2A mice (Figure 3C). Hence, 300 µM 5-MeO-DMT increased extracellular 5-HT to 149.5±22.1% of baseline in WT mice and 100 µM 5-MeO-DMT decreased 5-HT to 38.8±8.1% of baseline in KO2A mice. Two-way ANOVA of normalized AUCs (Figure 3D) of the different experimental periods used revealed significant effects of 5-MeO-DMT (F(3,30)=6.17; p<0.03), genotype (F(1,10)=10.17; p<0.01) and genotype x treatment interaction (F(3,30)=5.94; p<0.03).

3.4. Antipsychotic drugs reversal of 5-MeO-DMT effect in mPFC of WT mice.

As previously showed in rats (Riga et al., 2014) we examined in WT mice whether the antipsychotic drugs haloperidol (HAL) and risperidone (RIS) could reverse the disruption alteration of mPFC activity induced by 5-MeO-DMT. Figure 4 shows the reversal of 5-MeO-DMT effects on LFCO by HAL and RIS. Two-way ANOVA analysis revealed a significant effect of the 5-MeO-DMT treatment (F(2,26)=109.76, p<0.00001), antipsychotic treatment (F(2,13)=4.62, p<0.05) and of 5-MeO-DMT x antipsychotics treatments interaction (F(4,26)=3.47, p<0.03). Post-hoc analysis revealed significant differences between baseline and 5-MeO-DMT and between 5-MeO-DMT+saline and 5-MeO-DMT+antipsychotic treatments.
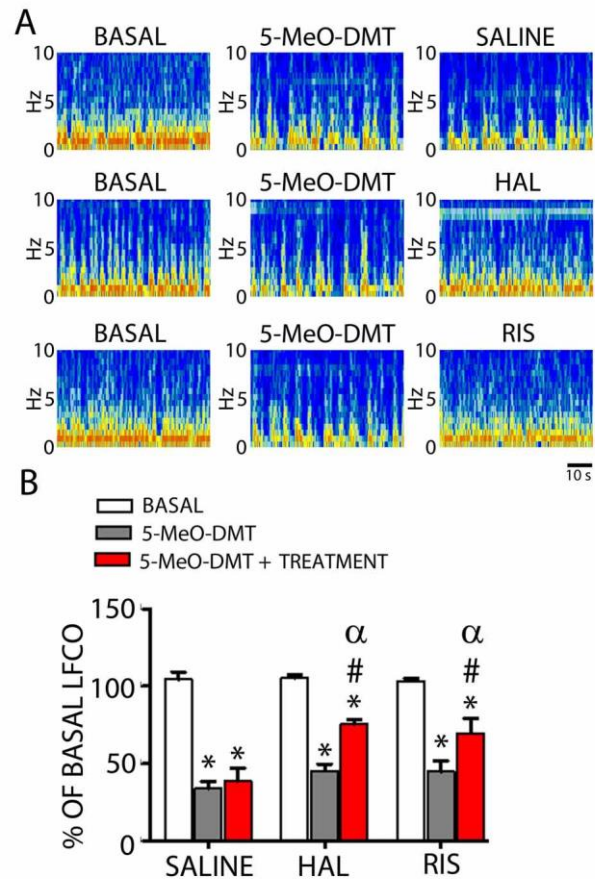
Figure 4. Reversal by antipsychotic drugs of the effects of 5-MeO-DMT (1 mg/kg subcutaneously) on low frequency cortical oscillations (LFCO). **A)** Spectrograms showing the effects of the administration of saline, haloperidol and risperidone on 5-MeO-DMT-induced reduction on LFCO. The intensity of the power spectrum is color-coded (red=high intensity; blue=low intensity). **B)** Bar graph showing the average effects on LFCO of 5-MeO-DMT + saline, (n=5); 5-MeO-DMT + haloperidol (0.6 mg/kg subcutaneously), (n=5) and 5-MeO-DMT + risperidone (1 mg/kg subcutaneously), (n=6). * p<0.003 *vs* basal; [#]p<0.01 *vs* 5-MeO-DMT; [α]p<0.002 *vs* 5-MeO-DMT + saline treatment.

3.5. Effect of 5-MeO-DMT on LFCO in sensory cortical areas of WT and KO2A mice.

To examine whether sensory cortical are affected by 5-MeO-DMT, we recorded LFCO in S1, Au1 or V1 using ECoGs. 5-MeO-DMT reduced LFCO in S1, Au1 and V1 (S1: 67.1±4.3%; Au1: 59.3%±4.1%; V1: 67.1±6.8% of baseline) of WT mice, but not in S1 and Au1 of KO2A mice. Interestingly, 5-MeO-DMT reduced LFCO in V1 of KO2A mice (50.2±5.1% of baseline). Figure 5 shows representative examples of the effect of 5-MeO-DMT on LFCO in S1, Au1 and V1 in the two genotypes. In WT mice, two-way ANOVA analysis revealed a significant effect of 5-MeO-DMT (F(1,30)=138.08; p<0.00001), with no effects of area and area x treatment interaction. In KO2A mice, two-way ANOVA analysis revealed a significant effect of 5-MeO-DMT

(F(1,22)=17.63; p<0.0005), area (F(2,22)=7.01; p<0.005) and area x treatment interaction (F(2,22)=7.61; p<0.005). Thus, 5-MeO-DMT disrupts differently S1-LFCO and Au1-LFCO in WT and KO-2A mice. On S1-LFCO two-way ANOVA analysis revealed a significant effect of 5-MeO-DMT (F(1,19)=12.17; p<0.003), genotype (F(2,22)=7.01; p<0.005) and genotype x treatment interaction (F(1,19)=6.18; p<0.03); on Au1-LFCO two-way ANOVA analysis revealed a significant effect of 5-MeO-DMT (F(1,14)=28.95; p<0.0001) and genotype x treatment interaction (F(1,14)=7.88; p<0.02). In contrast, two-way ANOVA analysis of LFCO in V1 revealed a significant effect of 5-MeO-DMT on LFCO (F(1,19)=12.17; p<0.003) with no effects of genotype and genotype x treatment interaction. Post-hoc analysis showed significant differences between 5-MeO-DMT treatment in S1 and Au1 in the two genotypes, but not in V1 (Figure 5B).
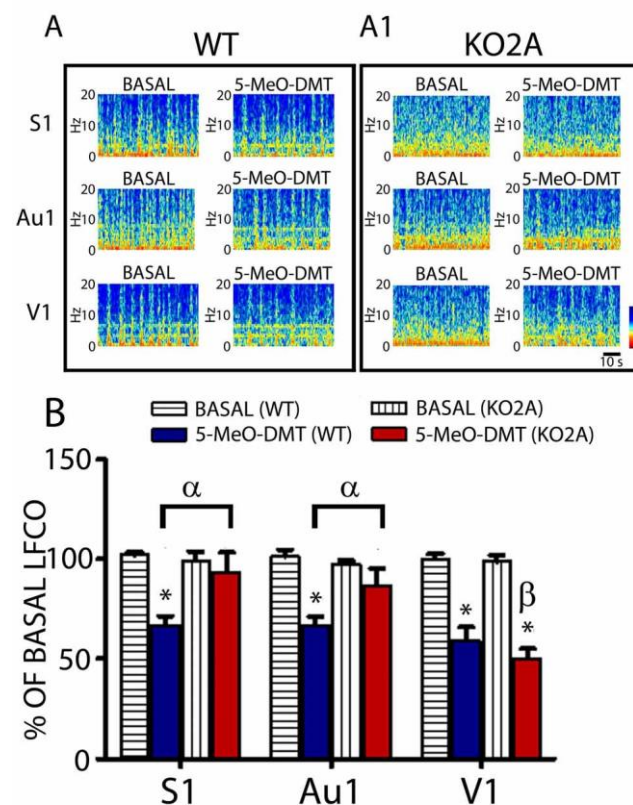

Figure 5. Effect of the administration of 5-MeO-DMT on the low frequency cortical oscillations (LFCO) in the primary somatosensory (S1) primary auditory (Au1) and primary visual (V1) cortices of WT and KO2A mice. **A)** and **A1)** Spectrograms showing the effect of 5-MeO-DMT in WT **A)** and KO2A **A1)** in a 1-min period before and 12 min after its administration. Time bars in abscissa are 10 s; ordinates are in Hz. The intensity of the power spectrum is color-coded (red = high intensity; blue = low intensity). Note the similar effect of 5-MeO-DMT in S1, Au1 and V1 in WT mice, the lack of 5-MeO-DMT effect in S1 and Au1 in KO2A mice and, curiously, the 5-MeO-DMT effect in V1 of KO2A mice. **B)** Bar graph showing the effects of 5-MeO-DMT on LFCO in WT and KO2A mice. * p<0.003 *vs* baseline; α p<0.01 5-MeO-DMT (WT *vs* KO2A); β p<0.001 5-MeO-

DMT in V1 *vs* S1 and Au1 (KO2A). WT mice, n=11, 10 and 12 for S1, Au1 and V1, respectively; KO2A mice, n=10, 6 and 9 for S1, Au1 and V1, respectively.

4. Discussion

The present study confirms and extends previous observations in rat brain, indicating that 5-MeO-DMT decreases LFCO in PFC by stimulating 5-HT$_{1A}$-R and 5-HT$_{2A}$-R. We also show that this effect is reversed by classical (haloperidol) and atypical antipsychotic drugs (risperidone). Moreover, in addition to PFC, 5-MeO-DMT reduced LFCO in primary sensory areas (S1, Au1 and V1) of WT -yet only in V1 of KO2A mice- supporting the involvement of 5-HT$_{1A}$-R in the visual alterations induced by 5-MeO-DMT. Overall, these observations shed further light on the neurobiological mechanisms involved in the brain areas/circuits related to psychotic symptoms, such as hallucinations.

Despite the interest of serotonergic hallucinogens as models of schizophrenia symptoms, few studies examined 5-MeO-DMT effects on brain activity (de Montigny and Aghajanian, 1977; Riba *et al*, 2002). In recent years, our group has characterized the reduction of LFCO in rodent PFC as a common trait of psychotomimetic agents, including PCP and serotonergic hallucinogens (Celada *et al*, 2008; Kargieman *et al*, 2007, 2012; Riga *et al*, 2014; see Celada *et al*, 2013 for review). These actions are countered by classical and atypical antipsychotic drugs.

The action of serotonergic hallucinogens has been attributed to the activation of 5-HT$_{2A}$-R, for which they show high affinity (Beique *et al*, 2007; Nichols, 2004; Gonzalez-Maeso *et al*, 2007; Vollenweider *et al*, 1998). However, behavioral studies with WAY-100635 and KO1A mice support the additional involvement of 5-HT$_{1A}$-R on the action of indolamine hallucinogens –and in particular 5-MeO-DMT (Halberstadt and Geyer 2011; Krebs-Thomson *et al*, 2006; Winter *et al*, 2000; Van den Buuse *et al*, 2011). In the pre-pulse inhibition (PPI) model, 5-HeO-DMT has opposite effects in rats (decrease; Krebs-Thomson *et al*, 2006) and mice (increase; Halberstadt and Geyer 2011), as observed for selective 5-HT$_{1A}$-R agonists (Gogos *et al*, 2008; Sipes and Geyer, 1995). Irrespectively of this species difference, the effect of 5-MeO-DMT on PPI was blocked or attenuated by the 5-HT$_{1A}$-R antagonist WAY-100635 (Halberstadt and Geyer 2011; Krebs-Thomson *et al*, 2006), supporting the involvement of 5-HT$_{1A}$-R.

5-MeO-DMT reduced LFCO and the BOLD signal in PFC and V1 of the rat. The fall in LFCO was prevented or reversed by selective 5-HT$_{1A}$-R and 5-HT$_{2A}$-R antagonists (Riga *et al*, 2014). Here, we extended the observations to mice and examined the involvement of 5-HT$_{1A}$-R and 5-HT$_{2A}$-R. Interestingly, 5-MeO-DMT reduced LFCO in the PFC of WT mice, as previously observed in rats (Riga *et al*, 2014). It also reduced LFCO in KO2A mice, yet to a smaller extent, which suggests an additional role for other 5-HT-R. Subsequent experiments indicated that WAY-100635 pretreatment prevented the 5-MeO-DMT-induced decrease of LFCO in KO2A mice. Overall, these observations indicate that 5-HT$_{1A}$-R and 5-HT$_{2A}$-R activation mediates the

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reduction in LFCO evoked by 5-MeO-DMT. Interestingly WAY-100635 enhanced LFCO in mPFC of KO2A mice but not in WT mice (Kargieman *et al*, 2012). The differential effect of WAY-100635 in WT and KO2A mice cannot be ascribed differences in 5-HT$_{1A}$-R density (Bortolozzi *et al*, 2010). More convincingly, given the high cellular co-expression and interactions between these receptors in PFC (see below), a functional compensatory change in the control of LFCO by 5-HT$_{1A}$-R may occur in KO2A mice.

5-MeO-DMT markedly reduced the discharge of 5-HT neurons (de Montigny and Aghajanian, 1977). Therefore, some of the observed changes might be due to the activation of presynaptic 5-HT$_{1A}$-R in the midbrain raphe and the subsequent reduction of 5-HT release in PFC. However, 5-HT$_{1A}$-R and 5-HT$_{2A}$-R in the mPFC also control serotonergic activity and the local 5-HT release via direct inputs to the raphe nuclei (Celada *et al*, 2001; Martín-Ruiz *et al*, 2001). These effects are due to the stimulation of 5-HT$_{1A}$-R and 5-HT$_{2A}$-R in pyramidal neurons (Santana *et al*, 2004) projecting to the DR (Gabbott *et al*, 2005; Vazquez-Borsetti *et al*, 2009). Hence, we examined the relative contribution of pre- and postsynaptic 5-HT$_{1A}$-R, by comparing the effects of 5-MeO-DMT on 5-HT release in PFC after systemic and local application.

The comparable reduction PFC 5-HT release in WT and KO2A mice after systemic 5-MeO-DMT administration (1 mg/kg s.c.) suggests a predominant role of presynaptic 5-HT$_{1A}$-R in this effect. Interestingly, the fall in 5-HT release was accompanied by an increase in the HTR in WT –not KO2A- mice indicating a parallel activation of postsynaptic 5-HT$_{2A}$-R at the dose used. On the contrary, local 5-MeO-DMT application in PFC evoked a differential concentration-response curve in WT and KO2A mice. At the lower concentration used (30 µM), 5-MeO-DMT evoked a similar reduction of the local 5-HT release in WT and KO2A mice, most likely due to the activation of 5-HT$_{1A}$-R in midbrain-projecting pyramidal neurons (Celada *et al*, 2001). The 5-HT reduction persisted in KO2A mice after the subsequent administration of higher 5-MeO-DMT concentrations (100 and 300 µM). However, local 5-MeO-DMT application evoked a concentration-dependent increase of 5-HT release in WT mice. The 5-HT increase in WT –not KO2A- mice is likely attributable to the activation of 5-HT$_{2A}$-R in PFC (Martín-Ruiz *et al*, 2001). These results suggest that 5-MeO-DMT acts preferentially on 5-HT$_{1A}$-R at low doses, occupying both receptors at higher doses. A limitation of these experiments is the difficulty to compare the activation of postsynaptic 5-HT-R produced by systemic and local 5-MeO-DMT administration. Despite of the nominal concentrations applied exceed the *in vitro* affinity of 5-MeO-DMT for the 5-HT-R several factors dramatically reduce the effective concentration once in the brain compartment. Thus, the passage of the dialysis membrane may reduce it by one order of magnitude and once in the extracellular compartment, 5-MeO-DMT is continuously cleared by the CSF. Finally, the reduced size of the dialysis membrane makes that only a small population of PFC neurons are affected.

As previously observed for PCP, DOI and 5-MeO-DMT in rats (Celada et al, 2008; Kargieman et al, 2007; Riga et al, 2014), the effects of 5-MeO-DMT were countered by antipsychotic drugs. The reversal by risperidone can be easily explained by direct displacement of 5-MeO-DMT from 5-HT2A-R. However, the

reversal by haloperidol needs to be interpreted at network level, since it shows low occupancy of 5-HT2A-R at the dose used (Schotte et al, 1993). Given the presence of dopamine D2-R in pyramidal and GABAergic neurons of mPFC (Santana et al, 2009), and their control of excitatory neurotransmission in PFC (Tseng and O'Donnell, 2007), D2-R blockade by HAL may normalize the excitatory/inhibitory balance altered by 5-MeO-DMT. Thus, antipsychotic drugs with different pharmacological profiles can equally restore the physiological state of LFCO, acting via different signalling pathways and/or cortical networks.

Various brain areas involved in the processing of sensory information show an altered activity in schizophrenia patients (Ford et al, 2014) as well as in healthy individuals and rodents treated with serotonergic hallucinogens (Kometer et al, 2011; Riga et al, 2014). In addition to PFC, 5-MeO-DMT reduced LFCO in S1, Au1 and V1 of WT mice and only in V1 of KO2A mice. Interestingly, the contribution of 5-HT$_{1A}$-R to the LFCO reduction differed among the cortical areas examined. Hence, the differential effect of 5-MeO-DMT in WT and KO2A mice was maximal in S1 and Au1, and minimal in V1, suggesting the preferential involvement of 5-HT$_{2A}$-R in Au1/S1 and of 5-HT$_{1A}$-R in V1.

5-HT$_{1A}$-R and 5-HT$_{2A}$-R are densely expressed in V1 (Dyck et al, 1993; Jakab et al, 1998) suggesting a central role of these receptors in visual processing. Interestingly, [3H]-5-HT labeled a dense population of 5-HT1 receptors (5-HT1A+5-HT1B+5-HT1D) in layer IVß of the human primary visual cortex (Pazos et al., 1987a). Similarly 5-HT2 receptors are also expressed in layer IVc in the same area (Pazos et al., 1987b). These observations suggest that both receptors are involved in the modulation of thalamic visual inputs from the lateral geniculate nucleus. To our knowledge, there are no similar detailed studies in the visual cortex of the rodent brain. Both receptors inhibit NMDA-induced LTP in visual cortex via different mechanisms (Jang et al, 2015 Kim et al, 2006). Interestingly, 5-HT$_{1A}$-R activation evokes plasticity phenomena in adult rats (Maya-Vetencourt et al, 2011). Thus, 5-HT$_{2A}$-Rs have been implicated in the pathogenesis of visual hallucinations (Ballanger et al, 2010) and both receptors participate in the sensory alterations evoked by psilocybin (Vollenweider et al, 1998; Carter et al, 2007). Likewise, the marked effect of 5-MeO-DMT in V1 found in the present study is consistent with the changes evoked by this drug on visual processing (de Araujo et al, 2012). However, the exact reason for the preferential action of 5-MeO-DMT on 5-HT1A-R in V1 is not fully understood.

It may appear contradictory that the activation of excitatory (5-HT$_{2A}$-R) and inhibitory (5-HT$_{1A}$-R) receptors contribute to reduce LFCO. However, there is a complex interplay between both receptors in PFC, which are expressed in pyramidal and GABAergic interneurons (Santana et al, 2004) and show a high cellular co-expression and functional interaction (Amargós-Bosch et al, 2004). Hence, despite endogenous 5-HT, released at PFC sites by the electrical stimulation of the DR, inhibit pyramidal neuron activity through activation of 5-HT$_{1A}$-R (Amargós-Bosch et al, 2004; Puig et al, 2005), the systemic administration of 5-HT$_{1A}$-R agonists increases pyramidal neuron discharge (Díaz-Mataix et al., 2006; Hajos et al, 1999; Lladó-Pelfort et al, 2012), an effect likely due to the preferential activation of 5-HT$_{1A}$-R in GABAergic interneurons (Lladó-

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Pelfort *et al*, 2012). This effect could add to the excitatory effects of 5-HT$_{2A}$-R activation (Puig et al., 2003), resulting in a synergistic interaction between both receptors. Thus, the above regional differences may depend on the proportion of 5-HT$_{2A}$-R and 5-H$_{1A}$-R in pyramidal and GABAergic neurons in the different cortical areas examined.

5. Conclusions

The present data indicate that the indoleamine hallucinogen 5-MeO-DMT evokes marked alterations in the function of primary sensory areas (Au1, S1, V1) as well as in the highest association cortex (PFC). These alterations are mediated by 5-HT1A-Rs and 5-HT2A-Rs, with a differential contribution of each receptor in the various areas examined. Thus, 5-HT1A-Rs play a major role on 5-MeO-DMT effect on visual and prefrontal cortices. These observations help to elucidate the neurobiological basis of hallucinations. Moreover, as previously observed with other pychotomimetic agents (PCP, DOI), the fall in LFCO induced by 5-MeO-DMT was countered by antipsychotic drugs, supporting the usefulness of the reversal of psychotomimetic effects on LFCO in antipsychotic drug development.

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