

Clozapine Reverses Phencyclidine-Induced Desynchronization of Prefrontal Cortex through a 5-HT_{1A} Receptor-Dependent Mechanism

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The non-competitive NMDA receptor (NMDA-R) antagonist phencyclidine (PCP)—used as a pharmacological model of schizophrenia—disrupts prefrontal cortex (PFC) activity. PCP markedly increased the discharge rate of pyramidal neurons and reduced slow cortical oscillations (SCO; 0.15–4 Hz) in rat PFC. Both effects were reversed by classical (haloperidol) and atypical (clozapine) antipsychotic drugs. Here we extended these observations to mice brain and examined the potential involvement of 5-HT_{2A} and 5-HT_{1A} receptors (5-HT_{2A}R and 5-HT_{1A}R, respectively) in the reversal by clozapine of PCP actions. Clozapine shows high *in vitro* affinity for 5-HT_{2A}R and behaves as partial agonist *in vivo* at 5-HT_{1A}R. We used wild-type (WT) mice and 5-HT_{1A}R and 5-HT_{2A}R knockout mice of the same background (C57BL/6) (KO-1A and KO-2A, respectively). Local field potentials (LFPs) were recorded in the PFC of WT, KO-1A, and KO-2A mice. PCP (10 mg/kg, intraperitoneally) reduced SCO equally in WT, KO-2A, and KO-1A mice (58 ± 4%, 42 ± 7%, and 63 ± 7% of pre-drug values, *n* = 23, 13, 11, respectively; *p* < 0.0003). Clozapine (0.5 mg/kg, intraperitoneally) significantly reversed PCP effect in WT and KO-2A mice, but not in KO-1A mice nor in WT mice pretreated with the selective 5-HT_{1A}R antagonist WAY-100635. The PCP-induced disorganization of PFC activity does not appear to depend on serotonergic function. However, the lack of effect of clozapine in KO-1A mice and the prevention by WAY-100635 indicates that its therapeutic action involves 5-HT_{1A}R activation without the need to block 5-HT_{2A}R, as observed with clozapine-induced cortical dopamine release.

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INTRODUCTION

The prefrontal cortex (PFC) plays a fundamental role in higher brain functions (Fuster, 1997; Miller and Cohen, 2001). Numerous observations suggest an abnormal function of this cortical area in schizophrenia (Elvevag and Goldberg, 2000; Goldman-Rakic, 1994; Lewis and Anderson, 1995; Uhlhaas and Singer, 2010; Uhlhaas and Singer, 2006; Winterer *et al*, 2004). In particular, anatomical, cellular, and neurochemical alterations have been reported in the frontal

lobe of patients with schizophrenia (Harrison, 1999; Lewis and Lieberman, 2000; Lewis *et al*, 2005; Selemon and Goldman-Rakic, 1999).

Non-competitive NMDA receptor (NMDA-R) antagonists such as phencyclidine (PCP) or ketamine exacerbate clinical symptoms in patients with schizophrenia and induce behavioral alterations that resemble schizophrenia symptoms in healthy individuals and experimental animals (Handelmann *et al*, 1987; Hudzik and Wenger, 1993; Jentsch and Roth, 1999; Krystal *et al*, 1994; Krystal *et al*, 2003; Pradhan, 1984; Stefani and Moghaddam, 2005; Verma and Moghaddam, 1996). In particular, acute PCP administration to humans induces a schizophrenia-like state (Allen and Young, 1978; Bakker and Amini, 1961; Castellani *et al*, 1982; Javitt and Zukin, 1991; Luby *et al*, 1959; Steinpreis, 1996). Overall, these observations have led to the use of these agents as pharmacological models of schizophrenia (Javitt and Zukin, 1991; Krystal *et al*, 1994; Malhotra *et al*, 1997; Newcomer *et al*, 1999). Clozapine (CLZ) the prototype

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of atypical antipsychotic drugs attenuates the behavioral and cognitive impairments induced by the acute administration of non-competitive NMDA-R antagonists (Geyer *et al*, 2001; Idris *et al*, 2005; Lipina *et al*, 2005).

Previous results indicate that acute PCP administration induces a profound loss of cortical synchrony at low frequencies (slow cortical oscillations, SCO; 0.15–4 Hz), an effect reversed by the subsequent administration of classical (haloperidol) and atypical (CLZ) antipsychotic drugs (Kargieman *et al*, 2007). PCP also exerts similar alterations in thalamic nuclei interconnected with the PFC, which are also reversed by CLZ administration (Santana *et al*, 2011). Interestingly, the hallucinogen DOI, a preferential 5-HT_{2A} receptor (5-HT_{2AR}) agonist, induced a comparable loss of SCO in PFC, an effect also reversed by haloperidol and CLZ (Celada *et al*, 2008). Overall, these observations suggest that the reduction in SCO and its reversal by antipsychotic drugs may be a useful model to identify new targets in antipsychotic drug development.

5-HT_{1A} receptor (5-HT_{1AR}) and 5-HT_{2A}R are abundantly expressed in rodent PFC (Pompeiano *et al*, 1992, 1994; Santana *et al*, 2004), where they are mostly co-expressed (Amargos-Bosch *et al*, 2004). They mediate opposing actions (inhibitions—5-HT_{1AR}; excitations—5-HT_{2AR}) of 5-HT and selective agonists (Amargos-Bosch *et al*, 2004; Araneda and Andrade, 1991; Marek and Aghajanian, 1999; Puig *et al*, 2005). CLZ shows an affinity for 5-HT_{2AR} greater than that for dopamine D2 receptors (Meltzer, 1999). Likewise, despite showing moderate *in vitro* affinity for 5-HT_{1AR}, CLZ and other atypical antipsychotic drugs behave as agonists at 5-HT_{1AR} *in vivo* to increase PFC dopamine release (Bortolozzi *et al*, 2010; Diaz-Mataix *et al*, 2005; Ichikawa *et al*, 2001; Rollema *et al*, 1997).

In this study, we examined whether PCP also alters SCO in mouse PFC. Moreover, taking advantage of the availability of mice lacking 5-HT_{1AR} and 5-HT_{2AR}, we further examined the involvement of such serotonin (5-HT) receptors in the mechanism of action of PCP and CLZ in the above-mentioned effects on SCO.

MATERIALS AND METHODS

Animals and Treatments

C57BL/6 (wild-type, WT) mice were used. Male homozygous 5-HT_{1AR} knockout (hereafter referred as KO-1A) mice and male homozygous 5-HT_{2A} receptor knockout mice (hereafter referred as KO-2A) were also used. Both strains were on a C57/BL6 background. Generation of both KO strains has been reported elsewhere (Fiorica-Howells *et al*, 2002; Parks *et al*, 1998; Weisstaub *et al*, 2006). From these initial sources, mice were transferred to the animal facility of the University of Barcelona School of Medicine, where stable colonies were grown. Animals were kept in a controlled environment (12 h light–dark cycle and 22 ± 2 °C room temperature) with food and water provided *ad libitum*. Animal care followed the European Union regulations (OJ of EC L358/1 18/12/1986) and was approved by the Institutional Animal Care and Use Committee.

PCP and CLZ were from Sigma/RBI (Natick, MA). Doses are expressed as free bases. Mice were injected intraperitoneally with the following treatments: (a) PCP (10 mg/kg),

followed by CLZ (0.5 mg/kg); (b) PCP (10 mg/kg), followed by saline; and (c) saline, followed by CLZ (0.5 mg/kg). Time between injections was 10–12 min. The dose of CLZ was chosen from the literature owing to its ability to antagonize behavioral effects of NMDA-R antagonists (Bradford *et al*, 2010; Gleason and Shannon, 1997; Mutlu *et al*, 2011; Scorza *et al*, 2010; Yadav *et al*, 2011).

To further evaluate the role of the 5-HT_{1AR} on CLZ-induced reversal of PCP actions on PFC, we performed an additional experiment in which mice were pretreated with saline or the selective 5-HT_{1AR} antagonist WAY-100635 (0.3 mg/kg, subcutaneously) 30 min before CLZ administration, as used previously (Duvvuri *et al*, 2009).

At the end of the experiments, mice were killed by anesthetic overdose and the placement of recording electrodes verified histologically.

Local Field Potential Recordings

We examined drug effects on local field potential (LFP). LFP recordings provide a measure of population neuronal activity around the tip of the recording electrode (Schroeder and Lakatos, 2009; Shoham and Nagarajan, 2003) and allow to assess oscillatory electrical activity.

Mice were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally) and positioned in a David Kopf stereotaxic frame. Additional doses of chloral hydrate (40 mg/kg) were administered intraperitoneally. Body temperature was maintained at 37 °C with a heating pad. LFPs were recorded with glass micropipettes pulled from 2 mm capillary glass (World Precision Instruments, Sarasota, FL) on a Narishige (Tokyo, Japan) PE-2 pipette puller. The electrode impedance was between 4 and 8 MΩ. The signal was amplified (×10) with a Neurodata IR283 (Cygnus Technology, Delaware Water Gap, PA), post-amplified (×100) and filtered using a band-pass filter (0.1–100 Hz) (Cibertec amplifier/filter, Madrid, Spain), and computed online using a DAT 1401plus interface system Spike2 software (CED). Descents in the medial PFC (mPFC) were carried out at AP +2.2 to +2.4, L −0.2 to −0.4, and DV −1.0 to −2.5 below brain surface. Stereotaxic coordinates were taken from bregma and duramater according to the mouse brain atlas (Franklin and Paxinos, 1997). After recording stable baseline activity for 5 min, PCP (10 mg/kg in saline) was slowly (15 s) administered intraperitoneally. CLZ (0.5 mg/kg, intraperitoneally) was injected 10 min after PCP administration. At the end of experiments, mice were killed by an overdose of anesthetic.

Data Analysis

The effect of PCP was examined in WT (*n* = 23), KO-1A (*n* = 11), and KO-2A (*n* = 13) mice. Off-line analysis was performed using the SPIKE 2 software (Cambridge Electronic Design, Cambridge, UK). Power spectra were constructed by using fast Fourier transformation of 1-min signal intervals (band-pass filter of 0.1–100 Hz) corresponding to baseline, PCP, and PCP + CLZ. Power resolution was 0.15 Hz. Analyses of drug effects on SCO (0.15–4 Hz) and on the dominant wave (~1 Hz) of SCO were measured separately. Results are given as AUCs.

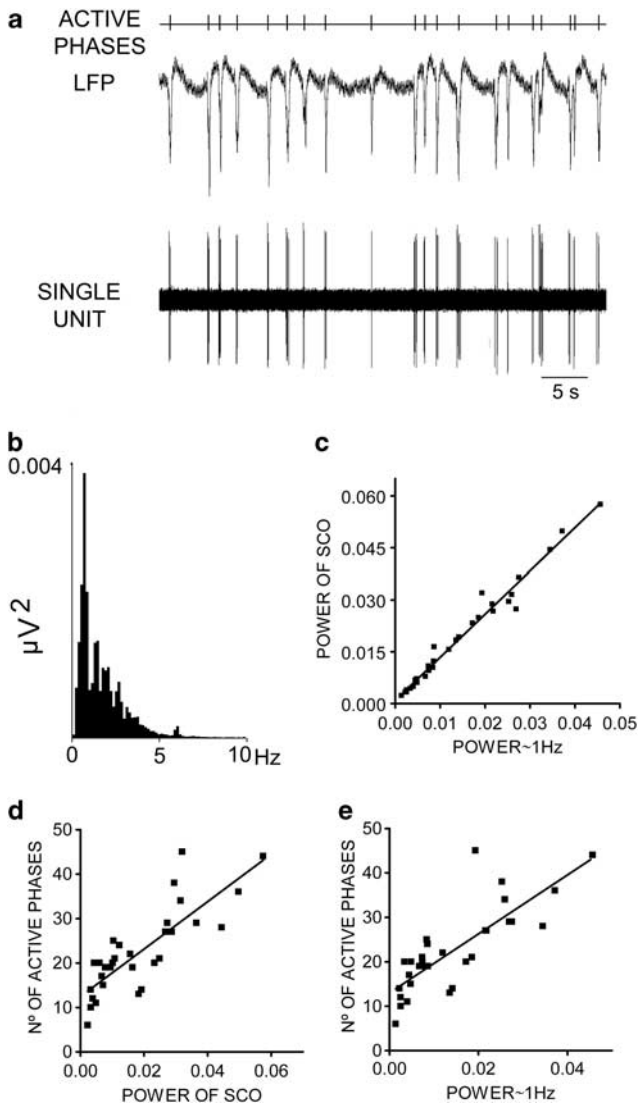


Figure 1 (a) Lower trace and mid-trace show action potential recorded extracellularly and local field potential (LFP) in mouse prefrontal cortex (PFC) in basal conditions. The upper trace depicts the number of UP states (see Materials and methods). Time bar is 5 s. (b) Power spectrum of the example is shown in (a). Note the predominance of the oscillation close to 1 Hz. (c–e) Correlations found between the individual basal values of three variables used in this study, (c) shows the correlation between the area under the curves (AUCs) of the power spectra of the 1 Hz oscillation (abscissa) with that of slow cortical oscillations (SCO) (0.15–4 Hz) (ordinate) ($r = 0.99$; $n = 31$). (d and e) show, respectively, the correlations between the AUCs of SCO and of the 1 Hz oscillation (abscissa) with the number of UP states (ordinate) ($r = 0.82$ and 0.81 , respectively; $n = 31$).

In addition, we measured the number of active phases in LFP recordings using a script of the Spike2 software. As observed in Figure 1a in basal conditions, the discharge of PFC pyramidal neurons occurs only during the active phase of the LFP, corresponding to depolarized or 'UP' states recorded intracellularly (Steriade *et al*, 1993a,b). Each downward deflection in the LFP trace was considered an active (or UP) phase when the time between two adjacent events was > 250 ms (eg, < 4 Hz; upper limit of SCO), and the amplitude of the deflection was three times the amplitude of the inactive phase. The actual recordings, as well as

the number of active (UP) phases, transcribed as events, are shown in Figure 1a.

The power spectra and the number of active phases were analyzed in 1-min time periods corresponding to baseline conditions (immediately before PCP administration), after PCP (10 min post-administration), and PCP + CLZ (10 min after CLZ administration). These time windows were selected on the basis of the behavioral effects of intraperitoneal administration of PCP, which start 3–4 min after injection and last for more than 20 min, as observed in non-anesthetized mice. Drug effects were analyzed using ANOVA, followed by *post-hoc* *t*-tests (Newman–Keuls) or paired Student's *t*-test, as appropriate. Statistical significance was set at the 95% confidence level (two-tailed). Data are given as means \pm SEM.

RESULTS

Characteristics of SCO in Mouse mPFC

LFP recordings of mouse mPFC in baseline conditions show the presence of characteristic SCO (0.15–4 Hz) with a marked predominance of a sharp wave at ~ 1 Hz (Figure 1). As observed in other species (Kargieman *et al*, 2007; Mukovski *et al*, 2007; Steriade *et al*, 1993b), action potentials were discharged in temporal coincidence with active phases of LFP (Figure 1a). Individual differences in SCO and in the ~ 1 Hz oscillations were paralleled by changes in the number of active phases. Significant correlations were found between the basal values of these variables: (i) SCO (0.15–4 Hz) vs 1 Hz oscillation ($r = 0.99$; $p < 0.0001$); (ii) SCO (0.15–4 Hz) vs number of active (UP) phases ($r = 0.82$; $p < 0.0001$); and (iii) 1 Hz oscillation vs number of active (UP) phases ($r = 0.81$; $p < 0.0001$) ($n = 31$; Figure 1). The high correlation between the individual values of SCO and the 1 Hz oscillation indicates that the latter wave is the main contribution to SCO in mice PFC.

The power spectra of SCO did not differ between genotypes (WT: 0.024 ± 0.003 ; KO-1A: 0.016 ± 0.003 ; and KO-2A: $0.017 \pm 0.004 \mu V^2$; ($F(2, 44) = 1.67$, $p = 0.20$), $n = 23$, 11, and 13, respectively). Likewise, the power spectra of the 1 Hz oscillation (WT: 0.018 ± 0.002 ; KO-1A: 0.011 ± 0.002 ; and KO-2A: $0.012 \pm 0.003 \mu V^2$; $n = 23$, 11, and 13, respectively) and the number of active phases per min (WT: 25.83 ± 1.81 ; KO-1A: 22.9 ± 3.31 ; and KO-2A: 18.54 ± 2.68 ; $n = 23$, 11, and 13, respectively) did not significantly differ among genotypes ($F(2, 44) = 2.55$, $p = 0.09$) and ($F(2, 44) = 2.44$, $p = 0.1$) for 1 Hz oscillation and the number of active phases per minute, respectively. The three variables (power spectra of SCO and ~ 1 Hz wave as well as number of active phases) were equally sensitive to drug effects (see below).

Effect of PCP on mPFC Oscillations

As previously reported in rats (Kargieman *et al*, 2007), the systemic administration of PCP to WT mice reduced SCO, from 0.024 ± 0.003 to $0.013 \pm 0.002 \mu V^2$ ($58.4 \pm 4.1\%$ of baseline; $p < 0.00001$; paired Student's *t*-test, $n = 23$). This reduction was paralleled by a similar decrease in the number of active phases (from 25.8 ± 1.8 to 14.1 ± 1.1 ; 54.6% of baseline; $p < 0.000001$, Student's *t*-test, $n = 23$). Figure 2 shows a representative example of the effect of PCP

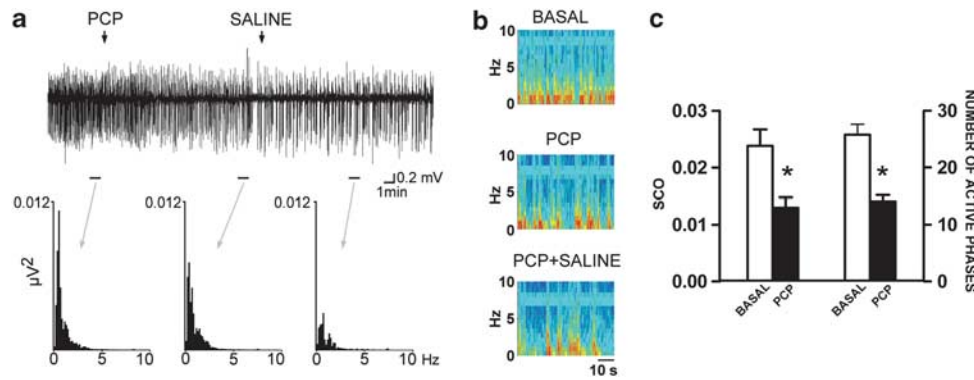


Figure 2 Effect of phencyclidine (PCP) administration on slow cortical oscillations (SCO). (a) Local field potential (LFP) recording and power spectra of a representative experiment showing the decrease in SCO after PCP administration. Small bars below the LFP recording denote the one 1-min period corresponding to the power spectrums shown below. Note that PCP effect persisted for at least 25 min. (b) Spectrograms showing the effects of the administration of PCP and saline in the time periods shown in (a). Time bar 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 PCP on SCO, and on the number of active phases per minute. * $p < 0.00001$ vs baseline; $n = 23$.

on SCO as well as the average effects on power spectra and number of active phases.

To examine the kinetics of PCP effect on SCO in mice, we measured the effect of PCP at 10 and 20 min post-administration in six experiments. Changes in PFC activity started at approximately 4 min after intraperitoneal administration of PCP and persisted for at least 20 min (Figures 2 and 3). One-way repeated-measures ANOVA of the power spectra of SCO revealed a significant effect of PCP at 10 and 20 min after administration (0.025 ± 0.007 , 0.017 ± 0.006 , and $0.012 \pm 0.004 \mu V^2$ in basal conditions and 10 and 20 min after PCP, respectively) ($(F(2, 10) = 9.47, p < 0.005)$, $n = 6$), with no significant differences between 10 and 20 min (Figures 2a and 3). Similarly, there were no differences between the number of active phases at 10 and 20 min (22.8 ± 3.3 , 13.5 ± 3.3 , and 12.7 ± 3.3) events per min in basal conditions and after 10 and 20 min of PCP administration, respectively ($(F(2, 10) = 18.56, p < 0.0004)$, $n = 6$; Figure 3).

Saline injections did not alter SCO, 1 Hz oscillations, nor the number of active phases (data not shown). Also, saline injections did not modify the SCO suppression induced by the subsequent PCP administration (Figure 2).

Effect of PCP on mPFC Oscillations in KO-1A and KO-2A Mice

Systemic PCP administration reduced SCO (0.15–4 Hz) similarly in the mPFC of WT, KO-1A, and KO-2A mice (Figures 2 and 4). Two-way ANOVA revealed a significant effect of PCP ($(F(1, 44) = 50.00, p < 0.0001)$, $n = 23, 11$, and 13 for WT, KO-1A, and KO-2A mice, respectively), with no significant effects of genotype and treatment \times genotype interaction.

The analyses of the effect of PCP on the 1 Hz oscillation also revealed a reduction of the power spectra in all genotypes. Two-way ANOVA revealed a significant effect of PCP ($(F(1, 44) = 38.87, p < 0.0001)$, $n = 23, 11$, and 13 for WT, KO-1A, and KO-2A mice, respectively), with no significant effects of genotype and treatment \times genotype interaction.

As in WT mice, PCP administration also reduced the number of active phases in the mPFC of KO-1A and KO-2A

mice, from 22.9 ± 3.3 to 13.2 ± 2.8 events per min (KO-1A, $p < 0.001$; Student's t -test, $n = 11$) and from 18.54 ± 2.68 to 7.85 ± 1.13 events per min (KO-2A, $p < 0.001$; Student's t -test, $n = 13$). Two-way ANOVA revealed a significant effect of PCP ($(F(1, 44) = 126.05, p < 0.0001)$, $n = 23, 13$, and 11 for WT, KO-2A and KO-1A mice, respectively) and genotype ($(F(2, 44) = 3.51, p < 0.05)$), but no significant effect of treatment \times genotype interaction (Figure 4).

CLZ Reversal of PCP Effect

Next, we next examined whether (i) CLZ could reverse PCP effects on SCO in the PFC of WT mice, as observed in rats (Kargieman *et al*, 2007), and (ii) whether CLZ effects were comparable in all genotypes (WT, KO-1A, and KO-2A).

WT Mice

As observed in rats (Kargieman *et al*, 2008), CLZ (0.5 mg/kg, intraperitoneally) had no effect by itself on SCO in WT mice (113% of basal values; NS, $n = 4$).

In the WT mice treated with PCP + CLZ ($n = 19$), PCP reduced SCO to $59.9 \pm 4.4\%$ of baseline, an effect significantly reversed by CLZ administration (Figure 5). One-way repeated-measures ANOVA revealed a significant effect of treatment ($(F(2, 36) = 30.82; p < 0.00001)$, with significant *post-hoc* differences between PCP vs baseline and PCP + CLZ vs PCP, indicating that CLZ reversed PCP effects on SCO in mouse PFC.

When the effects of PCP and CLZ were assessed by the change of the ~ 1 Hz wave or the number of active phases, the results were similar. Thus, PCP reduced the ~ 1 Hz wave to $55.4 \pm 4.7\%$ of baseline, an effect reversed by CLZ ($(F(2, 36) = 35.22; p < 0.00001)$, $n = 19$; one-way repeated-measures ANOVA), with significant *post-hoc* differences between PCP vs baseline and PCP + CLZ vs PCP (Figure 5). Likewise, CLZ reversed the PCP-evoked reduction in the number of active phases ($(F(2, 36) = 22.11; p < 0.00001)$, $n = 19$; one-way ANOVA), with significant *post-hoc* differences between PCP vs baseline and PCP + CLZ vs PCP (Figure 5).

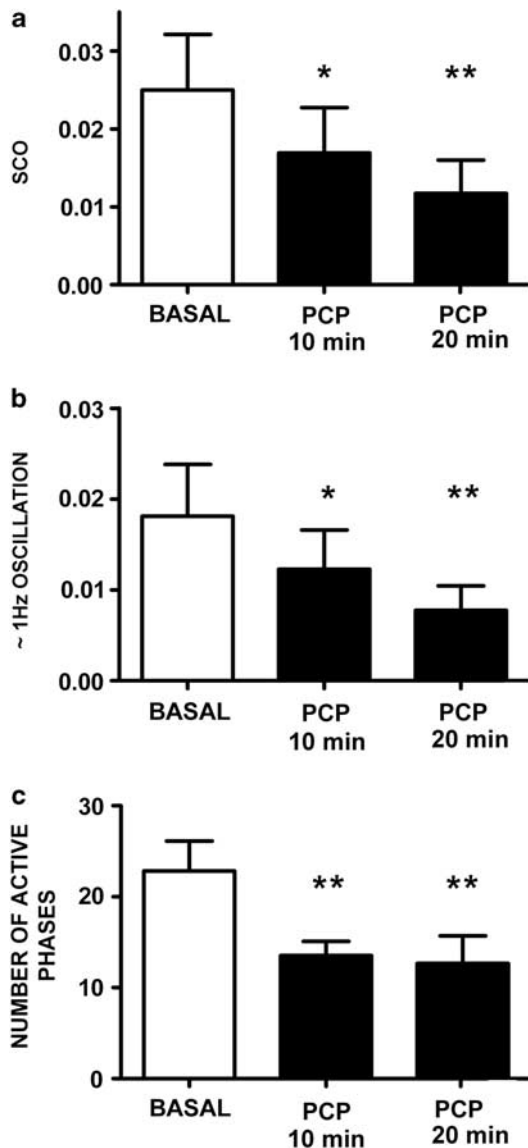


Figure 3 Bar graphs showing the effects of phencyclidine (PCP) on slow cortical oscillations (SCO) (a), on the ~1 Hz oscillation (b), and on the number of active phases per minute (c) 10 and 20 min after administration. * $p < 0.05$, ** $p < 0.005$ vs basal.

Genotype Comparisons

As observed in WT mice, the administration of CLZ (0.5 mg/kg, intraperitoneally) significantly reversed PCP effects on SCO in KO-2A mice, but failed to do it in KO-1A mice (Figure 6). Two-way ANOVA (treatment, genotype) analysis of the SCO data in all genotypes revealed a significant effect of treatment ($F(2, 78) = 39.9$, $p < 0.0001$) and significant treatment \times genotype interaction ($(F(4, 78) = 13.09$, $p < 0.00001$), $n = 19$, 11, and 12 for WT, KO-1A, and KO-2A mice, respectively). *Post-hoc* comparisons showed significant differences between PCP vs baseline activity in all genotypes and significant *post-hoc* differences between PCP + CLZ treatment vs treatment with PCP alone in WT mice and in KO-2A mice, but not in KO-1A mice (Figure 6).

Likewise, on the number of active phases, two-way ANOVA revealed a significant effect of treatment ($F(2, 78) = 71.07$, $p < 0.0001$), genotype ($F(2, 39) = 9.13$, $p < 0.001$), and treatment \times genotype interaction ($(F(4, 78) = 15.29$; $p < 0.00001$), $n = 19$, 11, and 12 for WT, KO-1A, and KO-2A mice, respectively), with significant *post-hoc* differences between PCP vs baseline in all genotypes and significant *post-hoc* differences between PCP + CLZ vs PCP in WT mice and in KO-2A mice. On the contrary, *post-hoc* comparisons showed that CLZ administration did not significantly reverse the effect of PCP in KO-1A mice (Figure 6). So, CLZ significantly overturned PCP effect in WT and KO-2A mice, but not in KO-1A mice.

Prevention of CLZ Effect in WT Mice by the 5-HT_{1A}R Antagonist WAY-100635

Pretreatment with the 5-HT_{1A}R antagonist WAY-100635 (0.3 mg/kg, subcutaneously) prevented the CLZ reversal of PCP-evoked reduction on SCO without having any effect on SCO by itself. Two-way ANOVA revealed a significant effect of treatment ($F(3, 30) = 23.76$, $p < 0.001$) and of the treatment \times pretreatment interaction ($(F(3, 30) = 3.88$, $p < 0.02$), $n = 6$ for saline and WAY-100635 pretreatment, respectively), with significant *post-hoc* differences between PCP vs baseline in both groups. Likewise, Newman-Keuls *post-hoc* test revealed a significant difference in the effect of CLZ in saline and WAY-100635-pretreated mice (Figure 7).

Likewise, two-way ANOVA also revealed a significant effect of treatment ($F(3, 30) = 8.54$, $p < 0.0003$) and treatment \times pretreatment interaction ($F(3, 30) = 5.02$; $p < 0.01$) ($n = 6$ each group) on the number of active phases. As observed with power spectra, Newman-Keuls *post-hoc* test revealed a significant difference in the effect of CLZ in saline and WAY-100635-pretreated mice (Figure 6).

DISCUSSION

This study shows that PCP is able to alter SCO in mouse PFC, thus extending previous observations in rat PFC, indicating a general action of PCP on rodent brain. This allows the use of transgenic mice to examine the involvement of neurotransmitter receptors in drug effects. In this regard, an important and novel observation of this study is the requirement of 5-HT_{1A}R activation for the CLZ-mediated reversal of PCP effects on SCO, indicating that 5-HT_{1A}R play an important role in the therapeutic action of CLZ. Likewise, a new methodological contribution of this study is the use of the number of active (UP) phases as a new variable to examine drug effects on cortical synchrony.

These findings are consistent with previous reports of the effects of psychotomimetic agents (eg, PCP and DOI) on SCO in rat PFC (Celada et al, 2008; Kargieman et al, 2007) and thalamic nuclei projecting to PFC (Santana et al, 2011). Hence, PCP markedly alters SCO in the PFC of both rodent species (Kargieman et al, 2007; this study) and these effects are restored by classical (haloperidol) and atypical (CLZ) antipsychotic drugs (Kargieman et al, 2007).

Cortical neurons exhibit synchronous, hyperpolarized (resting or DOWN state), and plateau depolarized (UP state) membrane potential fluctuations during slow-wave

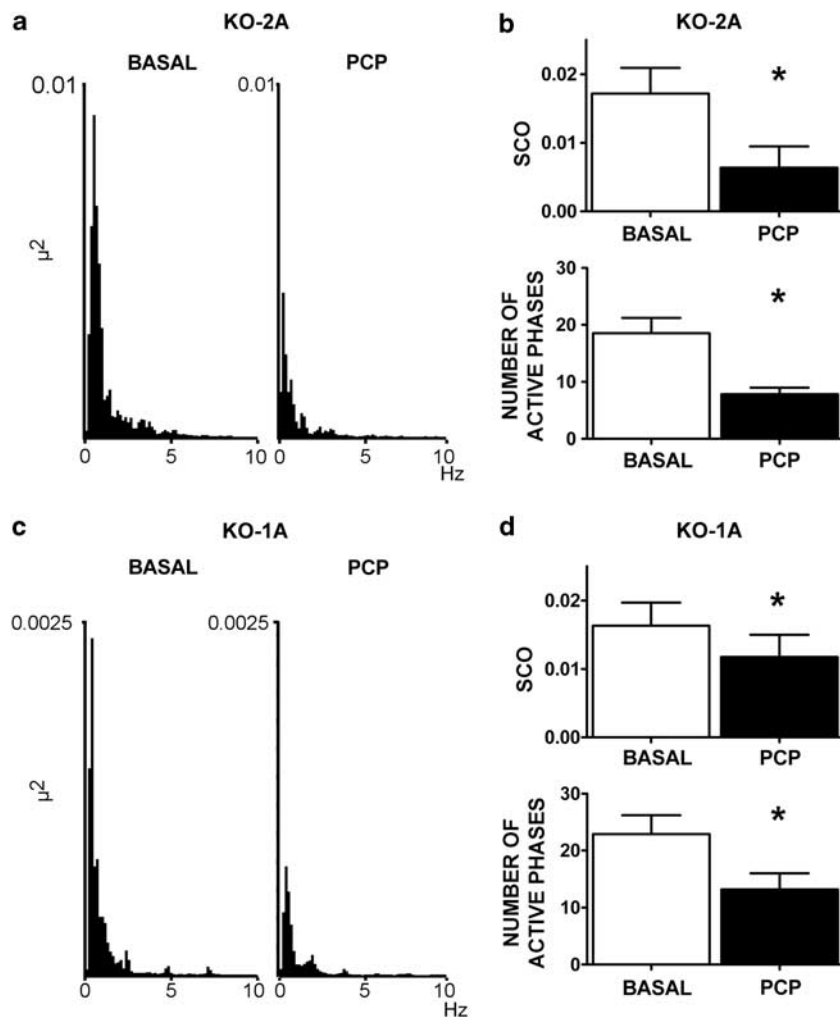


Figure 4 Effect of phencyclidine (PCP) on slow cortical oscillations (SCO) in the prefrontal cortex (PFC) of 5-HT_{2A} receptor (5-HT_{2A}R)- and 5-HT_{1A}R-knockout (KO) mice (KO-2A and KO-1A, respectively). Representative effect of PCP on SCO in the PFC of a KO-2A mouse (a) and a KO-1A mouse (c). Power spectrum show a decrease of SCO (0.15–4 Hz) after intraperitoneal (i.p.) PCP administration. Bar graph shows the effect of PCP on SCO and on the number of active phases per minute in KO-2A mice (b) and KO-1A mice (d). * $p < 0.002$; $n = 13$ and 11 , respectively.

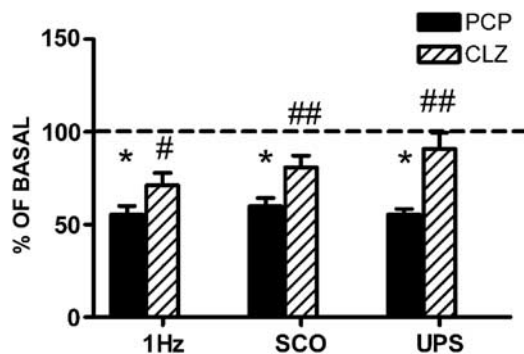


Figure 5 Reversal by clozapine (CLZ) of the phencyclidine (PCP)-induced reduction in slow cortical oscillations (SCO), ~1 Hz cortical oscillations, and number of active per minute. * $p < 0.0002$ vs basal, # $p < 0.01$ vs PCP, and ## $p < 0.0005$ vs PCP.

sleep and in anesthetized animals (Steriade *et al*, 1993a,b; Lewis and O'Donnell, 2000; Mukovski *et al*, 2007). These changes can be observed at the population level as SCO in

LFP and EEG recordings (Destexhe *et al*, 2007; Steriade *et al*, 1993b). The ~1-Hz cortical oscillation, predominant in SCO, appears to be essential for information processing in PFC (Engel *et al*, 2001; Engel and Singer, 2001). Hence, boosting low EEG frequencies during sleep has been shown to improve memory (Marshall *et al*, 2006), and the disruption of cortical network synchrony may have a negative impact on working memory and in behavioral representation, two important PFC-dependent tasks altered in schizophrenia (Elvevag and Goldberg, 2000).

The study of cortical oscillatory activity has a high translational value for the study of the pathophysiology and treatment of schizophrenia, as well as in the identification of neurobiological targets for drug development (Ford *et al*, 2007). Although most neurophysiological studies assessing oscillatory activity in schizophrenic patients have focused on high-frequency oscillations (β , γ) (Kwon *et al*, 1999; Spencer *et al*, 2003; Uhlhaas and Singer, 2006), alterations in lower frequencies have also been reported (Basar-Eroglu *et al*, 2009; Shin *et al*, 2010), which may be relevant to the emergence of schizophrenia symptoms given

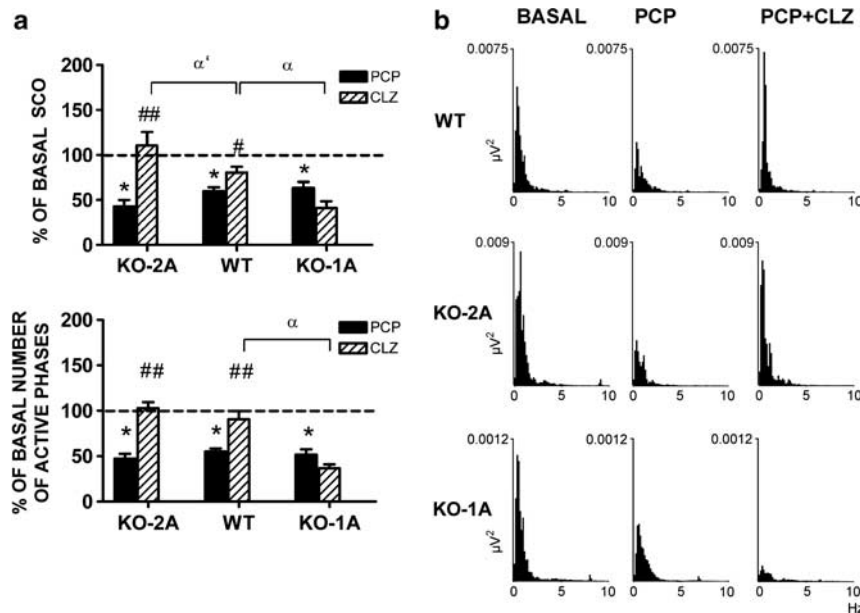


Figure 6 Reversal by clozapine of phencyclidine (PCP) effects on cortical oscillation in the three genotypes. (a) Graphs showing the average effects of PCP and PCP + clozapine (CLZ) as assessed by the area under the curves (AUCs) of power spectra (upper graph) and the number of active phases (lower graph). Note the lack of CLZ reversal in knockout (KO)-1A mice. $n = 23, 11$, and 12 for wild-type (WT), KO-1A, and KO-2A mice. * $p < 0.001$ vs basal; # $p < 0.05$ vs PCP; ## $p < 0.0005$ vs PCP; $\alpha p < 0.0002$, $\alpha p < 0.01$ post-hoc test after two-way analysis of variance (ANOVA) (see text). (b) Representative examples of the power spectra of slow cortical oscillations (SCO) in mice prefrontal cortex (PFC) obtained in basal conditions, after PCP and after the subsequent CLZ administration (10 and 20 min after PCP administration, respectively) in the three genotypes. Note the marked reduction of the power of SCO in the three genotypes by PCP and the reversal induced by CLZ in WT and KO-2A mice, but not in KO-1A mice.

the involvement of slow oscillations in cognitive processes and memory consolidation (Stickgold, 2005; Marshall *et al*, 2006).

Here we report that PCP administration markedly reduced synchrony in mouse PFC, as assessed by SCO (0.15–4 Hz), 1-Hz oscillations, and the number of active phases. These variables are correlated since they all reflect the synchronized alternating activity states of neuronal populations in the recording area and were equally sensitive to drug effects.

In normal conditions, action potentials are discharged synchronously in temporal coincidence with active (UP) phases (Kargieman *et al*, 2007; Mukovski *et al*, 2007; Steriade *et al*, 1993b; Figure 1), yet in the presence of PCP, cortical synchrony is markedly reduced or lost, whereas discharge rate is enhanced, leading to a profound temporal disorganization of PFC neuronal activity (Kargieman *et al*, 2007; this study).

The reduction of SCO and number of active phases is possibly due to an imbalance between excitatory and inhibitory neurotransmission within the PFC. In the rat PFC, PCP markedly increased (~ 3 -fold) the discharge rate of a 45% of pyramidal neurons and reduced it in another 33% (Kargieman *et al*, 2007). UP states are sustained by a balance of excitatory and inhibitory network interactions—including afferent modulatory inputs—and by intrinsic cellular mechanisms (Contreras and Steriade, 1995; Lewis and O'Donnell, 2000; O'Donnell and Grace, 1995; Sanchez-Vives and McCormick, 2000; Shu *et al*, 2003; Timofeev *et al*, 2000a, 2000b). Hence, the decrease in GABAergic activity reported in the NMDA-R model of schizophrenia (Gonzalez-Burgos and Lewis, 2008;

Homayoun and Moghaddam, 2007; Krystal *et al*, 2003; Lewis *et al*, 2005) may account for this effect, assuming a preferential blockade of NMDA-R in GABAergic interneurons by PCP. Thus, the blockade of GABA_A-mediated inhibitions with picrotoxin decreased the number of UP states (Cossart *et al*, 2003) and suppressed slow-wave oscillations (Shu *et al*, 2003). In support of this view, PCP mainly induced the expression of *c-fos*—a marker of neuronal activity—in cortical pyramidal neurons and on excitatory thalamic neurons, but not in GABA interneurons in PFC and subcortical areas reciprocally connected with PFC (Kargieman *et al*, 2007; Santana *et al*, 2011).

Overall, the present and past observations (Kargieman *et al*, 2007; Santana *et al*, 2011) indicate that PCP evokes a highly disorganized state in PFC, with an increased pyramidal discharge occurring in a random manner, without the temporal frame of the ~ 1 Hz oscillation, an effect occurring also in thalamic neurons reciprocally connected with PFC (Santana *et al*, 2011). This pattern of action is similar to that observed in recent human neuroimaging studies with ketamine (Vollenweider and Kometer, 2010). Given the top-down control exerted by PFC on most cortical and subcortical areas (Miller and Cohen, 2001), the disruption of PFC and thalamic function by PCP may have a profound impact on a large number of brain functions, which may account for the perceptual, cognitive, affective, and motor actions of PCP (Krystal *et al*, 1994, 2003).

PCP increases 5-HT release in PFC (Amargos-Bosch *et al*, 2006) and endogenous 5-HT can excite or inhibit pyramidal cell activity via 5-HT_{2A}R and 5-HT_{1A}R, respectively (Araneda and Andrade, 1991; Puig *et al*, 2005). The similar effects of PCP in all genotypes indicate that changes in SCO

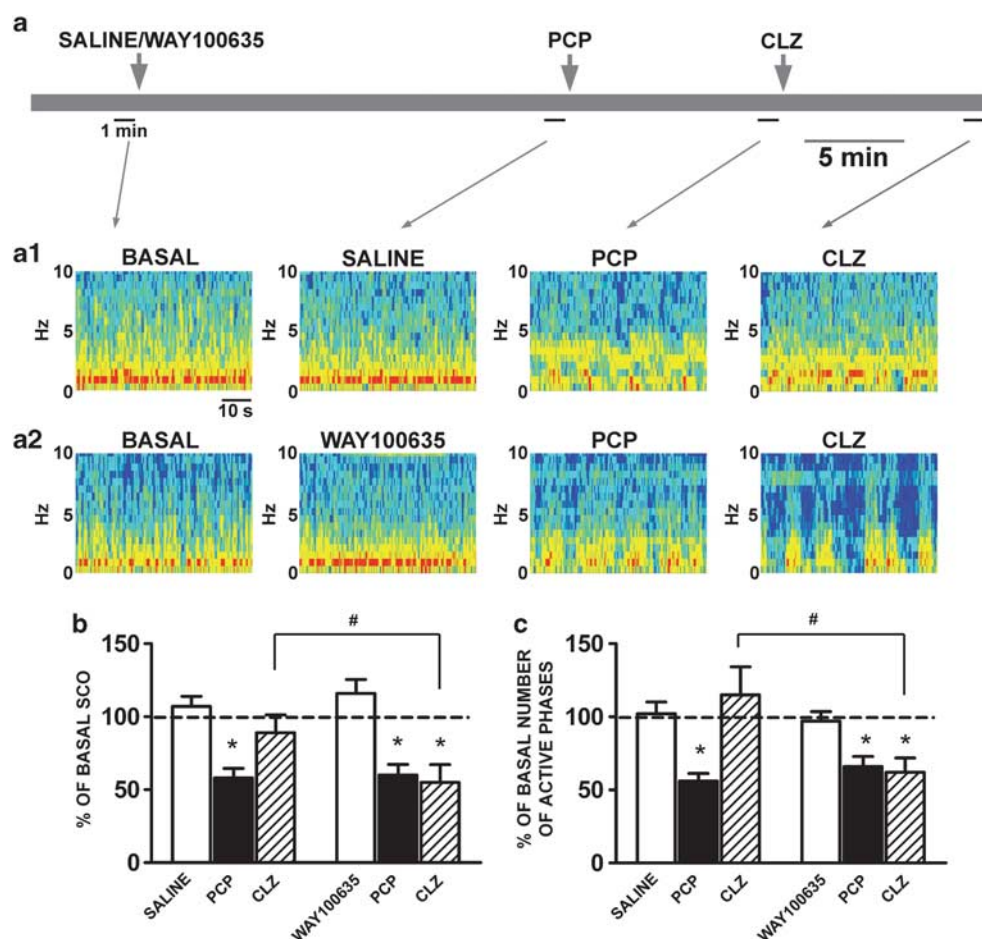


Figure 7 The 5-HT_{1A} receptor (5-HT_{1A}R) antagonist WAY-100635 prevents the clozapine (CLZ) reversal of phencyclidine (PCP) effect on slow cortical oscillations (SCO) in wild-type (WT) mice. (a) Representative examples of local field potential (LFP) recording in prefrontal cortex (PFC) from mice pretreated with saline (a1) and WAY-100635 (0.3 mg/kg, subcutaneously) (a2). Spectrograms showing the effects of the administration of WAY-100635 or saline, PCP, and CLZ in the time periods shown in the upper scheme. The intensity of the power spectrum is color-coded (red = high intensity; blue = low intensity) (b and c) Bar graph showing the prevention by WAY-100635 (0.3 mg/kg, subcutaneously) pretreatment of CLZ reversal of PCP-evoked reduction on SCO (b) and on the number of active phases per minute (c). WAY-100635 by itself has not effect on both parameters. **p* < 0.02 vs basal, #*p* < 0.01 saline-PCP-CLZ vs WAY-100635-PCP-CLZ.

occur upstream of the change in 5-HT release and without a significant involvement of such receptor populations.

In contrast, the effect of CLZ markedly differs among mouse genotypes. As in rat PFC, CLZ reversed PCP effect on SCO in WT and KO-2A mice. However, CLZ was without effect in KO-1A mice. Previous studies have demonstrated varying *in vitro* affinities of CLZ for 5-HT_{1A}R, for which it behaves as a partial agonist. Hence, CLZ displays a modest affinity for 5-HT_{1A}R (~100 nM) (Kroeze *et al*, 2003; Roth *et al*, 2004), whereas affinities in the range 700–800 nM were reported previously (Arnt and Skarsfeldt, 1998; Bymaster *et al*, 1996). Despite this moderate-low *in vitro* affinity, CLZ exerts a number of its *in vivo* pharmacological actions—particularly the increase in PFC DA release—via 5-HT_{1A}R activation (Bortolozzi *et al*, 2010; Diaz-Mataix *et al*, 2005; Ichikawa *et al*, 2001; Rollema *et al*, 1997), an observation that may have several interpretations.

5-HT_{1A}R and 5-HT_{2A}R are highly co-expressed in rat and mouse PFC neurons (Amargos-Bosch *et al*, 2004). Thus, although there are no compensatory changes of other 5-HT receptors in KO-1A and KO-2A mice (Amargos-Bosch *et al*,

2004; Bortolozzi *et al*, 2010; Popa *et al*, 2005), the blockade of 5-HT_{2A}R by CLZ might alter the physiological balance between 5-HT_{1A}R and 5-HT_{2A}R, resulting in an increase of 5-HT_{1A}R-mediated neurotransmission. However, these results, obtained in anesthetized mice, together with a recent report on the effect of CLZ on dopamine release in the PFC of awake mice (Bortolozzi *et al*, 2010) do not support this view, as CLZ reversed the effects of PCP on SCO in KO-2A mice, whereas it did not in KO-1A mice. Moreover, the observation that WAY-100635 pretreatment prevented the CLZ-induced reversal of PCP effects on SCO suggests a direct interaction of CLZ (or its main metabolite *N*-desmethylozapine) with 5-HT_{1A}R. This view agrees with PET scan data, indicating the partial displacement of [¹¹C]WAY-100635 (a hardly displaceable ligand) by CLZ in monkey brain (Chou *et al*, 2003). This apparent discrepancy between *in vitro* and *in vivo* data may be also accounted for by the contribution of the main CLZ metabolite, *N*-desmethylozapine, which shows 5-HT_{1A}R affinity in the 10 nM range (<http://www.kidb.case.edu/pdsp.php>).

The requirement of 5-HT_{1A}R activation by CLZ to restore SCO is entirely similar to its ability to enhance PFC dopamine release, an effect thought to be involved in the superior efficacy of CLZ on negative symptoms and cognitive deficits (Leucht *et al*, 2009a, b). Hence, CLZ and other atypical antipsychotic drugs (but not classical dopamine D2 blockers) share the ability to increase DA release in rodent mPFC through 5-HT_{1A}R activation (Bortolozzi *et al*, 2007; Diaz-Mataix *et al*, 2005; Ichikawa *et al*, 2001; Li *et al*, 2009; Rollema *et al*, 1997, 2000), an effect depending on the exclusive activation of 5-HT_{1A}R in mPFC as observed in KO mice (Bortolozzi *et al*, 2007, 2010; Diaz-Mataix *et al*, 2005). These results also agree with a recent report indicating that CLZ's ability to normalize NMDA-R hypofunction *in vivo* depends on an intact presynaptic serotonergic function and that 5-HT_{2A}R are not essential for CLZ reversal of PCP-induced disruption of sensory-motor gating (Yadav *et al*, 2011).

Irrespectively of the mechanisms involved, the requirement of 5-HT_{1A}R for CLZ to increase dopamine release in PFC and to restore SCO suggests an association between both effects that requires further investigation. Hence, the dopamine increase may restore the loss of SCO via activation of D1 receptors in fast-spiking interneurons (Tseng *et al*, 2006). In support of the involvement of GABA interneurons in the effect of CLZ, the combination of PCP and CLZ induced the expression of *c-fos* in PFC GABAergic neurons (Kargieman *et al*, 2007; Santana *et al*, 2011), suggesting that CLZ may restore cortical synchrony via an enhancement of GABA inputs onto pyramidal neurons excited by PCP.

In summary, this study shows for the first time that PCP markedly reduces cortical synchrony in mouse PFC. The experiments carried out in KO mice illustrate the validity of this model to examine the involvement of neurotransmitter receptors in drug effects and indicate that the actions of CLZ to restore cortical synchrony require the presence of 5-HT_{1A}R, as observed using other experimental models, such as the cortical release of dopamine (Bortolozzi *et al*, 2010). Overall, this adds further support to the involvement of this 5-HT receptor in antipsychotic drug action (Newman-Tancredi, 2010).

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DISCLOSURE

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