Published in final edited form as:

Psychopharmacology (Berl). 2013 January ; 225(1): 217–226. doi:10.1007/s00213-012-2809-7.

Persistent effects of chronic clozapine on the cellular and behavioral responses to LSD in mice

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Abstract

Rationale—In schizophrenia patients, optimal treatment with antipsychotics requires weeks to months of sustained drug therapy. However, single administration of antipsychotic drugs can reverse schizophrenia-like behavioral alterations in rodent models of psychosis. This raises questions about the physiological relevance of such antipsychotic-like activity.

Objective—This study evaluates the effects of chronic treatment with clozapine on the cellular and behavioral responses induced by the hallucinogenic serotonin 5-HT_{2A} receptor agonist lysergic acid diethylamide (LSD) as a mouse model of psychosis.

Method—Mice were treated chronically (21 days) with 25 mg/kg/day clozapine. Experiments were conducted 1, 7, 14, and 21 days after the last clozapine administration. [³H]Ketanserin binding and 5-HT_{2A} mRNA expression were determined in mouse somatosensory cortex. Head-twitch behavior, expression of *c-fos*, which is induced by all 5-HT_{2A} agonists, and expression of *egr-1* and *egr-2*, which are LSD-like specific, were assayed.

Results—Head-twitch response was decreased and [³H]ketanserin binding was downregulated in 1, 7, and 14 days after chronic clozapine. 5-HT_{2A} mRNA was reduced 1 day after chronic clozapine. Induction of *c-fos*, but not *egr-1* and *egr-2*, was rescued 7 days after chronic clozapine. These effects were not observed after short treatment (2 days) with clozapine or chronic haloperidol (1 mg/kg/day).

Conclusion—Our findings provide a murine model of chronic atypical antipsychotic drug action and suggest downregulation of the 5-HT_{2A} receptor as a potential mechanism involved in these persistent therapeutic-like effects.

Keywords

Schizophrenia; Hallucinogenic drugs; LSD; Mouse models; GPCR

Introduction

Schizophrenia is a severe and persistent psychiatric condition that affects more than 1 % of the population throughout the world (Sawa and Snyder 2002; Dobbs 2010). Second generation (or atypical) antipsychotic drugs, such as clozapine, olanzapine, and risperidone, are commonly used in the treatment of schizophrenia and other psychotic disorders (Lieberman et al. 2008). A single administration of antipsychotic drugs induces distinctive patterns of neuronal signaling and electrophysiological responses within minutes to hours (Wang and Liang 1998; Kontkanen et al. 2002b; Celada et al. 2008; Kargieman et al. 2011). Nevertheless, it is now well accepted that the therapeutic effects of anti-psychotic drugs start to be present only after the first days or weeks of treatment and accumulate over time (Freed 1988; Agid et al. 2003; Lieberman et al. 2008). Pharmacokinetic studies in humans demonstrate that the time to peak plasma concentrations of clozapine after single-dose administration ranges from 0.5 to 4 h (Jann et al. 1993; Lin et al. 1994), whereas its elimination half-life is about 12 h (Choc et al. 1987; Cheng et al. 1988). However, following abrupt withdrawal of chronic clozapine administration, the relapse in schizophrenia patients tends to occur within 1–2 weeks or more after the last dose (Borison et al. 1988). Together, these findings suggest that the time course of clozapine blood levels does not correlate with its therapeutic effects and that their acute effects are unlikely to underlie the therapeutic action of antipsychotic medications.

Because schizophrenia is a unique human disorder, animal models of schizophrenia and antipsychotic drug action have substantial limitations (Nestler and Hyman 2010). One group of putative pharmacological models of schizophrenia uses psychedelic drugs, such as phencyclidine (PCP) (Morris et al. 2005; Kristiansen et al. 2007) and lysergic acid diethylamide (LSD) (Nichols 2004; Gonzalez-Maeso and Sealfon 2009a), which produce psychotic symptoms and cognitive deficits similar to those observed in schizophrenic patients (Geyer and Vollenweider 2008; Gonzalez-Maeso and Sealfon 2009b). The manifestations of schizophrenia are divided into “positive” symptoms (e.g., hallucinations and delusions), negative symptoms (e.g., social withdrawal and apathy), and cognitive symptoms (e.g., deficits in attention, memory and executive functioning) (Sawa and Snyder 2002; Freedman 2003). Recent neuropsychological studies in healthy volunteers suggest that PCP-like drugs represent a proper model for schizophrenia negative symptoms together with certain positive symptoms, whereas LSD-like drugs elicits behavioral changes that have similarities with the positive symptoms in schizophrenic patients (Vollenweider et al. 1997, 1998; Gouzoulis-Mayfrank et al. 2005a, b). These results support the use of psychedelic drugs to model different aspects or types of schizophrenia and other psychotic disorders in rodents.

Atypical antipsychotic drugs all bind with high affinity to the serotonin 5-HT_{2A} receptor and a modest affinity to the dopamine D₂ receptor (Miyamoto et al. 2005; Lieberman et al. 2008). First generation (or typical) antipsychotic drugs, such as haloperidol and chlorpromazine, all have in common a high affinity for the dopamine D₂ receptor. Previous findings demonstrate that cortical serotonin 5-HT_{2A} receptor plays a key role in the mechanisms underlying the cellular and behavioral effects of LSD-like drugs (Beique et al.

2007; Gonzalez-Maeso et al. 2007; Celada et al. 2008). Drug-elicited head-twitch behavior is a useful model for studying hallucinogen activity at 5-HT_{2A} receptor in rodents (Gewirtz and Marek 2000; Garcia et al. 2007; Yadav et al. 2011a). Interestingly, it has been shown that the gene expression pattern induced by hallucinogenic drugs in somatosensory cortex is able to predict the presence or absence of head-twitch behavior in mouse (Gonzalez-Maeso et al. 2003, 2007). Thus, *c-fos* mRNA expression in somatosensory cortex results from intraperitoneal (i.p.) injection of 5-HT_{2A} agonists such as DOI, DOM, DOB, LSD, mescaline, psilocin, R-lisuride, S-lisuride, and ergotamine. However, only hallucinogenic drugs (i.e., DOI, DOM, DOB, LSD, mescaline, and psilocin) induce 5-HT_{2A} receptor-dependent head-twitch behavior and *egr-1* and *egr-2* mRNA expression in mouse somatosensory cortex (Gonzalez-Maeso et al. 2003, 2007). These findings suggest that hallucinogenic 5-HT_{2A} receptor-dependent induction of *egr-1* and *egr-2* in mouse somatosensory cortex may be a marker for hallucinogenic signaling.

Few paradigms have been able to consistently demonstrate modulation of cellular and behavioral responses after chronic, but not short-term, treatment with antipsychotic drugs. The present study examined whether chronic (21 days) treatment with clozapine differentially affects the behavioral (head-twitch) and cellular (expression of *c-fos*, *egr-1*, and *egr-2* in somatosensory cortex) induced by the hallucinogenic drug LSD in mice. In order to determine the persistent effects of chronic treatment with clozapine, the cellular and behavioral effects elicited by a single administration of LSD were investigated at different times (1, 7, 14, and 21 days) after the last clozapine injection. The correlation of 5-HT_{2A} receptor binding with the cellular and behavioral responses induced by LSD was tested. The effects of chronic treatment with clozapine were compared to those induced by short-term (2 days) treatment with clozapine or chronic treatment with the typical antipsychotic haloperidol.

Methods

Animals

Experiments were performed on adult (8–12 weeks old) male 129 S6/Sv mice. Animals were purchased from Taconic (Hudson, NY) and were housed at 12-h light/dark cycle (lights on, 8:00 to 20:00) at 23 °C with food and water ad libitum. The mice were housed for at least 1 week before experimental use. Four to five mice were housed per cage unless otherwise indicated. The Institutional Animal Use and Care Committee at Mount Sinai School of Medicine approved all experimental procedures (Academies 2011).

Materials and drug administration

Clozapine, methysergide, and haloperidol were obtained from Tocris Cookson Inc. (Minneapolis, MN, USA). Lysergic acid diethylamide (LSD) was purchased from Sigma-Aldrich (St. Louis, MO, USA). [³H]Ketanserin was purchased from PerkinElmer Life and Analytical Sciences Inc. (Branford, CT, USA). The injected doses (i.p.), calculated as salts, were clozapine, 1.5, 10.0, or 25.0 mg/kg; haloperidol, 1.0 mg/kg; and LSD, 0.24 mg/kg. These doses were selected based on previous findings with clozapine, haloperidol, and LSD in rodent models (Kuoppamäki et al. 1993; Kontkanen et al. 2002a, b; Gonzalez-Maeso et al. 2008; Gray et al. 2009). These doses represent approximately 12.5, 3.3, and 0.03 % of the LD₅₀ of clozapine (200 mg/kg), haloperidol (30 mg/kg), and LSD (800 mg/kg) after i.p. administration in mice, respectively. Clozapine was injected after suspension in a minimal amount of DMSO supplemented with acetic acid and made up to volume with normal saline. Haloperidol was dissolved in saline. LSD was dissolved in a minimal amount of DMSO and made up to volume with normal saline. Control animals received vehicle injections. Drugs

were administered in an injection volume of 100 μ l/10 g mouse body weight. All other chemicals were obtained from standard sources.

For effect of chronic treatment with clozapine on head-twitch behavior, chronic (21 days) treatment with clozapine started 3, 4, 5, and 6 weeks before the mice were injected with a single dose of LSD (i.e., 1, 7, 14, or 21 days after the last injection of chronic clozapine). Control mice were chronically (21 days) treated with vehicle and injected on the day of the experiment with a single dose of LSD (i.e., 1 day after the last injection of chronic vehicle). Injection of LSD was performed on the same day in all the groups of mice (i.e., acute LSD 1, 7, 14, or 21 after the last injection of chronic clozapine, and of acute LSD 1 day after the last injection of chronic vehicle).

For the effect of chronic treatment with clozapine on the expression of 5-HT_{2A} receptor (i.e., radioligand binding and mRNA), chronic (21 days) treatment with clozapine started 3, 4, 5, and 6 weeks before the mice were sacrificed (i.e., 1, 7, 14, or 21 days after the last injection of chronic clozapine). Control mice were chronically (21 days) treated with vehicle and sacrificed 1 day after the last injection of chronic vehicle. Mice were sacrificed on the same day in all the groups of mice (i.e., chronic clozapine 1, 7, 14, or 21 days after the last injection and chronic vehicle 1 day after the last injection).

For the effect of chronic treatment with clozapine on the LSD-dependent induction of *c-fos*, *egr-1*, and *egr-2* in mouse somatosensory cortex, treatment with clozapine or vehicle started 3, 4, 5, and 6 weeks before the mice were injected with a single dose of LSD or vehicle on the day of the experiment (i.e., 1, 7, 14, or 21 days after the last injection of chronic clozapine). Mice were sacrificed 60 min after injection with LSD. Similar conditions were used for chronic treatment with haloperidol. For short-term treatments, mice received one injection of clozapine, or vehicle, per day for 2 days, and experiments were performed 1 day after the last injection.

Head-twitch behavior

Head-twitch behavior was performed as previously reported (Gonzalez-Maeso et al. 2007). The head-twitch response induced by hallucinogenic 5-HT_{2A} receptor agonists has been shown to be affected by circadian rhythms, with maximum response at the end of the dark period (Singleton and Marsden 1981). Behavioral testing was conducted under dim light conditions (one 60 W light bulb illuminating the room, facing away from the animals). Experiments took place between 9:00 a.m. and 6:00 p.m. In order to avoid intraassay and interassay variations due to circadian influences, different experimental groups and appropriate controls were compared in assays conducted the same day within a 5-h time period. Videotape records were scored for head twitches by an experienced observer who was blinded to the antipsychotic treatment (i.e., clozapine, haloperidol, or vehicle).

Radioligand binding

Experiments were performed as previously reported (Gonzalez-Maeso et al. 2008).

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) experiments were performed as previously reported (Gonzalez-Maeso et al. 2007).

Statistical analysis

Radioligand binding data were analyzed using nonlinear curve fitting software (GraphPad Prism). An extra-sum-of-squares (*F* test) was used to determine statistical difference for simultaneous analysis of binding saturation curves. Statistical significance of biochemical or

behavioral experiments involving three or more groups and two or more treatments was assessed by two-way ANOVA, followed by Bonferroni's post hoc test. Statistical significance of biochemical or behavioral experiments involving three or more groups was assessed by one-way ANOVA, followed by Bonferroni's post hoc test. Statistical significance of biochemical or behavioral experiments involving two groups was assessed by Student's *t* test. The level of significance was chosen at $P=0.05$. All data are presented as mean $\pm$ SEM.

Results

Effect of chronic clozapine on the head-twitch behavior induced by LSD

We first investigated the effect of chronic treatment with clozapine on the head-twitch behavioral response induced by LSD. Previous studies have shown cellular and behavioral effects of chronic administration of clozapine in a dose range from 1.5 to 25 mg/kg (Kontkanen et al. 2002a, b; Gonzalez-Maeso et al. 2008). We first established the dose of clozapine required to induce persistent effects on the head-twitch behavioral response to LSD. Mice were chronically treated with clozapine (1.5, 10, or 25 mg/kg) or vehicle before assessing the LSD-dependent head-twitch response 1 and 7 days after the last clozapine injection. The head-twitch response to LSD was significantly reduced by chronic treatment with 1.5, 10, or 25 mg/kg clozapine on the day after the last injection. However, the head-twitch response to LSD was significantly reduced by chronic treatment with 25 mg/kg, but not 1.5 mg/kg, or 10 mg/kg of clozapine 7 days after the last injection. Experiment 1 consists of the following: vehicle, 41.75 ± 3.19 events/30 min, $n=4$; clozapine (1.5 mg/kg; 1 day after), 12.75 ± 4.69 events/30 min, $n=4$, $P<0.05$, one-way ANOVA followed by Bonferroni's post hoc test; and clozapine (1.5 mg/kg; 7 days after), 48.25 ± 10.48 events/30 min, $n=4$, $P>0.05$, one-way ANOVA followed by Bonferroni's post hoc test. Experiment 2 consists of the following: vehicle, 58.75 ± 3.19 events/30 min, $n=4$; clozapine (10 mg/kg; 1 day after), 4.01 ± 0.91 , $n=4$, $P<0.001$, one-way ANOVA followed by Bonferroni's post hoc test; and clozapine (1.5 mg/kg; 7 days after), 55.00 ± 2.81 events/30 min, $n=4$, $P>0.05$, one-way ANOVA followed by Bonferroni's post hoc test. Experiment 3 consists of the following: vehicle, 56.33 ± 8.92 events/30 min, $n=6$; clozapine (25 mg/kg; 1 day after), 6.33 ± 1.08 , $n=6$, $P<0.001$, one-way ANOVA followed by Bonferroni's post hoc test; and clozapine (25 mg/kg; 7 days after), 28.33 ± 5.56 events/30 min, $n=6$, $P<0.01$, one-way ANOVA followed by Bonferroni's post hoc test. These data show that the LSD-dependent head-twitch response is significantly reduced by chronic treatment with 25 mg/kg, but not 1.5 or 10 mg/kg, clozapine, 7 days after the last injection.

In the next group of experiments, mice were treated chronically with clozapine (25 mg/kg), or vehicle, before assessing the LSD-dependent head-twitch response 1, 7, 14, and 21 days after the last clozapine injection. One-way ANOVA indicated that there is a significant effect of chronic treatment with clozapine on the head-twitch response induced by LSD (Fig. 1a). Post hoc analysis revealed that head-twitch response was significantly reduced 1, 7, and 14 days after the last clozapine injection (Fig. 1a). Post hoc analysis also indicated that the head-twitch response induced by LSD was not affected 21 days after the last clozapine injection (Fig. 1a). The head-twitch response was not affected by short-term (2 days) treatment with clozapine (25 mg/kg/day, Fig. 1b), or chronic haloperidol (1 mg/kg/day, Fig. 1c).

Animal weight was not affected by chronic treatment: vehicle (i.e., control to clozapine): before chronic vehicle, 27.10 ± 0.69 g, $n=6$; 1 day after chronic vehicle, 27.10 ± 1.20 g, $n=6$. Clozapine (25 mg/kg) consisted the following: before chronic clozapine, 26.18 ± 0.59 g, $n=6$; 1 day after chronic clozapine, 26.08 ± 0.83 , $n=6$. Saline (i.e., control to haloperidol) consisted the following: before chronic saline, 25.01 ± 0.45 g, $n=6$; 1 day after chronic saline, $25.45 \pm$

0.89 g, $n=6$. Haloperidol (1 mg/kg) consisted the following: before chronic haloperidol, 25.98 ± 1.19 g, $n=6$; 1 day after chronic haloperidol, 25.54 ± 0.79 , $n=6$. Each dose of clozapine (25 mg/kg) induced hypolocomotor activity, an effect that was reduced as the chronic treatment progressed (data not shown). This effect was not observed 24 h after clozapine injection (data not shown).

Effect of chronic clozapine on 5-HT_{2A} receptor expression in mouse somatosensory cortex

The specific binding of the 5-HT_{2A} receptor antagonist [³H] ketanserin to mouse somatosensory cortex membrane preparations was a saturable process to a single population of high-affinity sites (Fig. 2a). The simultaneous analysis of multiple saturation curves indicated that chronic treatment with clozapine induces significantly different [³H]ketanserin-binding saturation curves ($F[8,259] = 117.4$; $P < 0.001$). Analysis of individual maximum number of binding sites ($B_{\max}$) values showed a lower density of 5-HT_{2A} receptor in mice sacrificed 1, 7, and 14 days, but not 21 days, after the last chronic injection of clozapine (Fig. 2b). The affinity (K_D values) for [³H]ketanserin binding did not differ from that obtained in mice chronically treated with vehicle (vehicle, 5.85 ± 1.07 nM; 1 day, 8.10 ± 2.74 nM; 7 days, 4.28 ± 0.96 nM; 14 days, 2.61 ± 0.58 nM; and 21 days, 4.52 ± 0.43 nM; $P > 0.05$). [³H]Ketanserin binding was not affected in mouse somatosensory cortex by short-term treatment with clozapine ($F[2,77] = 0.25$; $P > 0.05$; Figs. 2c, d) or chronic treatment with haloperidol ($F[2,55] = 0.34$; $P > 0.05$; Fig. 2e, f).

Chronic treatment with clozapine significantly downregulated the expression of *5-HT2A* mRNA in mouse somatosensory cortex 1 day after the last injection, with no changes 7, 14, and 21 days after the last injection ($F[4,63] = 3.63$, $P < 0.05$) (Fig. 2 g). The expression of *5-HT2A* mRNA was not affected by short-term treatment with clozapine (vehicle, 100.0 ± 3.67 %; short-term treatment with clozapine, 108.5 ± 4.26 %; $P > 0.05$ Student's *t* test; $n=12$) or chronic haloperidol (saline, 100.0 ± 3.86 %; chronic haloperidol, 98.5 ± 5.16 %; $P > 0.05$ Student's *t* test; $n=6$), 1 day after the last injection. Chronic treatment with clozapine does not affect the expression of *5-HT2C* or *D2* mRNA in mouse somatosensory cortex (data not shown).

Effect of chronic clozapine on the cellular responses induced by LSD

It has been previously demonstrated that hallucinogenic 5-HT_{2A} receptor agonists cause induction of *c-fos*, *egr-1*, and *egr-2* in mouse cortical neurons (Gonzalez-Maeso et al. 2003, 2007). Expression of *c-fos* is induced by all hallucinogenic and nonhallucinogenic drugs studied that activate the 5-HT_{2A} receptor. However, expression of *egr-1* and *egr-2* robustly predicts the hallucinogen-specific head-twitch behavior induced by hallucinogenic drugs such as LSD in rodents. Interestingly, induction of expression of *c-fos*, *egr-1*, and *egr-2* by LSD was significantly affected by the time after the last clozapine injection: expression of *c-fos*, $F(3,104) = 53.74$, $P < 0.001$; expression of *egr-1*, $F(3,83) = 29.68$, $P < 0.001$; and expression of *egr-2*, $F(3,97) = 103.40$, $P < 0.001$ (Table 1). Chronic treatment with clozapine eliminates the induction of *c-fos*, *egr-1*, and *egr-2* by LSD 1 day after the last injection (Table 1). The induction of *c-fos* was unaffected 7 and 14 days after the last injection with clozapine, whereas the induction of *egr-1* and *egr-2* by LSD was significantly attenuated (Table 1). No effect of chronic treatment with clozapine on the induction of *c-fos*, *egr-1*, and *egr-2* by LSD was observed 21 days after the last injection (Table 1). Expression of *c-fos*, *egr-1*, and *egr-2* was undistinguishable in somatosensory cortex of mice chronically treated with clozapine followed by acute vehicle as compared to mice chronically treated with vehicle followed by acute vehicle (Table 1). The induction of *c-fos*, *egr-1*, and *egr-2* by LSD was not affected by short-term treatment with clozapine (Table 2) or chronic haloperidol (Table 3).

Discussion

In this study, we show that chronic treatment with clozapine results in a persistent attenuation of the cellular and behavioral responses induced by LSD. We demonstrate that neither short-term treatment with clozapine nor chronic treatment with haloperidol affects these LSD-dependent effects. Evidence that 5-HT_{2A} receptor density as measured by [³H]ketanserin binding in mouse somatosensory cortex correlates with the hallucinogen-specific signaling signature has also been provided. It has previously been shown that regulation of *c-fos* in mouse somatosensory cortex tracks with agonist activity at the 5-HT_{2A} receptor, but expression of *egr-1* and *egr-2* robustly predicts the head-twitch behavior induced by hallucinogenic drugs (Gonzalez-Maeso et al. 2007). We found here that the density of [³H]ketanserin binding was significantly decreased (~30 %) 1 day after the last administration of chronic clozapine in mouse somatosensory cortex. Since cortical 5-HT_{2A} receptor is necessary for induction of *c-fos*, *egr-1*, and *egr-2* by LSD (Gonzalez-Maeso et al. 2007), downregulation of 5-HT_{2A} receptor density by chronic clozapine may be the mechanism responsible for this effect. We measured the degree of recovery of the density of [³H]ketanserin binding at different times after the last administration of chronic clozapine. By doing this, we found that partial recovery of [³H]ketanserin binding (~43 and ~58 % at days 7 and 14 after the last clozapine injection, respectively) was sufficient to induce significant expression of *c-fos* by LSD, yet induction of *egr-1* and *egr-2* by LSD remained decreased. Full recovery of [³H]ketanserin binding 21 days after the last administration of clozapine (~88 %) was necessary to induce the hallucinogen-specific expression of *egr-1* and *egr-2* in mouse somatosensory cortex. More importantly, this signaling pattern perfectly correlates with the head-twitch behavior induced by LSD. In mice, the head-twitch response is a specific acute behavioral response to hallucinogens interacting with the 5-HT_{2A} receptor. Pharmacologically and structurally related nonhallucinogenic drugs that also activate this receptor do not elicit the head-twitch response (Gonzalez-Maeso et al. 2003, 2007). These findings in this current study show that the head-twitch response was significantly reduced 1, 7, and 14 days after the last clozapine administration and not affected 21 days after the last injection as compared to mice chronically treated with vehicle followed by single administration of LSD. Since LSD-like drugs induce positive psychosis-like symptoms in healthy volunteers (Vollenweider et al. 1997, 1998; Gouzoulis-Mayfrank et al. 2005a, b), these findings suggest persistent antipsychotic-like effects induced by chronic, and not short-term, treatment with antipsychotic drugs. Neither LSD-dependent expression of *c-fos*, *egr-1*, and *egr-2* in mouse somatosensory cortex nor head-twitch behavior was affected by chronic treatment with the typical antipsychotic haloperidol. Although further investigation is necessary with additional typical and atypical antipsychotic drugs, these findings suggest that chronic treatment with atypical antipsychotic drugs induce prolonged effects on the cellular and behavioral responses induced by hallucinogenic 5-HT_{2A} agonists as a mouse model of therapeutic-like effects.

Expression of the immediate early genes *c-fos*, *egr-1*, and *egr-2* was not affected in somatosensory cortex of mice chronically treated with clozapine followed by acute saline as compared to mice chronically treated with vehicle followed by acute saline. Previous findings suggest effects of chronic treatment with clozapine and olanzapine on the expression of these genes (Kontkanen et al. 2002a; MacDonald et al. 2005; Verma et al. 2006). These divergent results may be due to mouse strain differences, the use of qRT-PCR assays rather than immunocytochemistry as primary method to study immediate early gene expression, or brain region-specific differences.

Single administration of clozapine attenuates some of the cellular and behavioral effects induced by PCP-like and LSD-like psychedelic drugs (Schreiber et al. 1994; Hitchcock et al. 1997; Wang and Liang 1998; Ichikawa et al. 2001; Celada et al. 2008; Bradford et al. 2010).

However, antipsychotic drugs often decrease the basal locomotor activity at the same doses at which they reduce the behavioral responses induced by PCP-like and LSD-like drugs (Sams-Dodd 1998). Similarly, single administration of 5-HT_{2A} receptor antagonists, such as M100907 and ritanserin, also abolishes some of the psychosis-like behavioral responses induced by LSD-like drugs (Willins and Meltzer 1997; Ouagazzal et al. 2001; Smith et al. 2003; Winter et al. 2005), yet they lack antipsychotic properties in schizophrenic patients (Gray and Roth 2007). Together with the 5-HT_{2A} receptor, clozapine and other atypical antipsychotic drugs bind with high affinity to other subtypes of monoaminergic receptors (Roth et al. 2004; Miyamoto et al. 2005). This polyreceptor affinity of atypical antipsychotics, and the effect of nonantipsychotic 5-HT_{2A} receptor antagonists on the behavioral responses induced by psychedelic drugs, make the results obtained in behavioral experiments after a single administration of clozapine difficult to interpret. In rodents, the half-life of clozapine and other antipsychotics is only 1.5 h (Cheng and Paalzow 1992; Baldessarini et al. 1993) (see above for additional information in schizophrenia patients). Although further pharmacokinetic studies are needed to determine the presence or absence of clozapine in the CNS, these findings suggest that the presence of clozapine is not necessary for the cellular (induction of *c-fos*, *egr-1*, and *egr-2*) and behavioral (head-twitch) effects induced by a single administration of LSD 3 weeks (~336 half-life periods) after the last clozapine injection. These findings also suggest that prolonged downregulation of the 5-HT_{2A} receptor by chronic, and not short-term, treatment with clozapine may be, at least in part, responsible for the antipsychotic-like suppression of the cellular and behavioral responses induced by hallucinogens. This hypothesis is further supported by prior studies examining the effect of chronic treatment with antipsychotics, 5-HT_{2A} receptor antagonists, and 5-HT_{2A} receptor inverse agonists on 5-HT_{2A}-like immunoreactivity in frontal cortex and modulation of the modulation of the PCP-induced hyperlocomotor activity in mice (Yadav et al. 2011b).

Recent evidence suggests epigenetic changes and chromatin structural modifications associated with chronic anti-psychotic drug action (Dong et al. 2008). Reduced dendritic spine density on cortical pyramidal neurons has been shown in postmortem human brain of schizophrenic subjects (Glantz and Lewis 2000; Black et al. 2004) and in mouse models of schizophrenia (Gilmore et al. 2004). Signaling downstream of the 5-HT_{2A} receptor modulates synaptic morphology and function (Jones et al. 2009). Based on these findings, additional research will be necessary to understand the role of the 5-HT_{2A} receptor in these and other therapeutic-like effects induced by chronic treatment with atypical antipsychotic drugs.

Acknowledgments

NIMH 5R01MH084894 (J.G.M.), NIDA P01 DA12923 (S.C.S.), NARSAD (J.G.M.), the Mortimer D. Sackler Foundation Award (J.G.M.), and Dainippon Sumitomo Pharma (J.G.M.) participated in the funding of this study.

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Fig. 1.

a Animals were chronically (21 days) treated with clozapine (25 mg/kg/day), or vehicle, and the head-twitch response induced by single administration of LSD (0.24 mg/kg) was determined 1, 7, 14, and 21 days after the last chronic injection ($n=4-5$ per group). *Single asterisk* $P<0.05$; *triple asterisk* $P<0.001$; *n.s.*, not significant; Bonferroni's post hoc test of one-way ANOVA. **b** Animals were treated (2 days) with clozapine (25 mg/kg/day), or vehicle, and the head-twitch response induced by single administration of LSD (0.24 mg/kg) was determined 1 day after the last injection ($n=4-5$ per group). **c** Animals were chronically (21 days) treated with haloperidol (1 mg/kg/day), or vehicle, and the head-twitch response induced by single administration of LSD (0.24 mg/kg) was determined 1 day after the last chronic injection ($n=4-5$ per group). *n.s.*, not significant; Student's t test. Data are presented as mean $\pm$ SEM

Fig. 2.

a [3H]Ketanserin-binding saturation curves in mouse somatosensory cortex of mice chronically (21 days) treated with clozapine (25 mg/kg/day) or vehicle. Experiments were carried out 1, 7, 14, and 21 days after the last chronic injection ($n=6$ per group). **b** [3H]Ketanserin binding obtained from individual saturation curves normalized as a percentage maximum number of binding sites (B_{max}) in control mice. *Single asterisk* $P<0.05$; *triple asterisk* $P<0.001$; *n.s.*, not significant; Bonferroni's post hoc test of one-way ANOVA. **c** [3H]Ketanserin-binding saturation curves in mouse somatosensory cortex of mice treated (2 days) with clozapine (25 mg/kg/day), or vehicle, and sacrificed 1 day after the last injection ($n=6$ per group). **d** [3H]Ketanserin binding obtained from individual saturation curves normalized as a percentage maximum number of binding sites (B_{max}) in control mice. *n.s.*, not significant; Student's *t* test. **e** [3H]Ketanserin-binding saturation curves in mouse somatosensory cortex of mice chronically (21 days) treated with haloperidol (1 mg/kg/day), or vehicle, and sacrificed 1 day after the last injection ($n=6$ per group). **f**

[³H]Ketanserin binding obtained from individual saturation curves normalized as a percentage maximum number of binding sites ($B_{\max}$) in control mice. *n.s.*, not significant; Student's *t* test. **g** Expression of *5-HT_{2A}* mRNA in mouse somatosensory cortex assayed by qRT-PCR. Animals were injected chronically (21 days) with clozapine (25 mg/kg/day), or vehicle, and sacrificed 1, 7, 14, and 21 days after the last injection ($n=12$ per group). *Double asterisk* $P<0.01$; Bonferroni's post hoc test of one-way ANOVA. Data are presented as mean $\pm$ SEM

Table 1

Effect of chronic treatment with clozapine on the LSD-dependent expression of *c-fos*, *egr-1*, and *egr-2* in mouse somatosensory cortex

	1 day			7 days			14 days			21 days		
	<i>c-fos</i>	<i>egr-1</i>	<i>egr-2</i>	<i>c-fos</i>	<i>egr-1</i>	<i>egr-2</i>	<i>c-fos</i>	<i>egr-1</i>	<i>egr-2</i>	<i>c-fos</i>	<i>egr-1</i>	<i>egr-2</i>
V + V	1.00±0.14	1.00±0.11	1.00±0.15	1.00±0.05	1.00±0.01	1.00±0.04	1.00±0.13	1.00±0.12	1.00±0.06	1.00±0.28	1.00±0.05	1.00±0.26
V + LSD	2.72±0.22 ***	1.63±0.09 ***	3.53±0.34 ***	2.32±0.09 ***	1.66±0.13 **	3.62±0.25 ***	2.51±0.20 ***	1.70±0.23 **	3.62±0.62 ***	2.54±0.15 ***	1.61±0.12 ***	3.57±0.27 ***
Clz + V	1.28±0.20	0.97±0.07	1.24±0.16	0.98±0.14	0.92±0.07	0.81±0.10	1.23±0.36	0.80±0.09	1.10±0.31	1.01±0.12	0.92±0.13	1.02±0.14
Clz + LSD	1.27±0.11	1.04±0.05	1.29±0.14	2.14±0.22 ***	1.09±0.09	1.48±0.10	2.22±0.21 **	1.10±0.09	1.97±0.14	2.41±0.18 ***	1.62±0.24 **	3.21±0.22 ***

Mice were chronically (21 days) treated (i.p.) with vehicle (V) or clozapine (Clz, 25 mg/kg/day), injected with a single dose of vehicle or LSD (0.24 mg/kg) at different times (1, 7, 14, or 21 days) after the last administration of chronic clozapine and were sacrificed after 60 min. Relative fold changes of mRNA expression are shown ($n=7-12$ per group). Bonferroni's post hoc test of two-way ANOVA. Data are presented as mean ± SEM

* $P<0.05$;

** $P<0.01$;

*** $P<0.001$

Table 2

Effect of short-term treatment with clozapine on the LSD-dependent expression of *c-fos*, *egr-1*, and *egr-2* in mouse somatosensory cortex

	<i>c-fos</i>	<i>egr-1</i>	<i>egr-2</i>
V + V	1.00±0.08	1.00±0.01	1.00±0.05
V + LSD	2.59±0.18 ***	1.61±0.05 **	3.35±0.30 **
Clz + V	0.90±0.08	0.93±0.07	0.79±0.17
Clz + LSD	2.35±0.16 ***	1.59±0.21 *	4.04±0.79 **

Mice were injected (2 days) with vehicle (V) or clozapine (Clz, 25 mg/kg/day), injected with a single dose of vehicle or LSD (0.24 mg/kg) 1 day after the last administration of clozapine and were sacrificed after 60 min. Relative fold changes of mRNA expression are shown ($n=4-6$ per group). Bonferroni's post hoc test of one-way ANOVA. Data are presented as mean $\pm$ SEM

* $P<0.05$;

** $P<0.01$;

*** $P<0.001$

Table 3

Effect of chronic treatment with haloperidol on the LSD-dependent expression of *c-fos*, *egr-1*, and *egr-2* in mouse somatosensory cortex

	<i>c-fos</i>	<i>egr-1</i>	<i>egr-2</i>
V + V	1.00±0.10	1.00±0.04	1.00±0.17
V + LSD	2.40±0.21 ***	1.65±0.17 *	2.91±0.62 *
Hal + V	0.95±0.15	1.02±0.10	0.81±0.05
Hal + LSD	2.61±0.19 ***	1.64±0.16 **	3.35±0.49 *

Mice were chronically (21 days) treated (i.p.) with vehicle (V) or haloperidol (Hal, 1 mg/kg/day), injected with a single dose of vehicle or LSD (0.24 mg/kg) 1 day after the last administration of chronic haloperidol and were sacrificed after 60 min. Relative fold changes of mRNA expression are shown ($n=6-10$ per group). Bonferroni's post hoc test of one-way ANOVA. Data are presented as mean $\pm$ SEM

* $P<0.05$;

** $P<0.01$;

*** $P<0.001$