Orbitofrontal cortex neurons as a common target for classic and glutamatergic antipsychotic drugs

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Until recently, all known antipsychotic drugs were thought to block the dopamine D2 receptor. New evidence that agonists of the metabotropic glutamate 2/3 (mGlu2/3) receptors ameliorate psychotic and affective symptoms of schizophrenia suggests that compounds with different molecular targets may act on a common cellular target to treat schizophrenia. We hypothesized that normalizing the activity of neurons in the orbitofrontal cortex (OFC), a region that is increasingly implicated in the pathophysiology of schizophrenia, presents such a target. We disrupted OFC activity in behaving rats with a use-dependent NMDA antagonist to model the NMDA hypofunction state that may occur in schizophrenia. This systemic treatment increased the activity of most pyramidal cells while inhibiting the activity of putative inhibitory GABA interneurons and increasing behavioral stereotypy. A similar pattern of OFC firing disruption was observed after amphetamine, which models a dopamine hyperactivity state in schizophrenia and which produces a pattern of firing disruption different from those of NMDA antagonists in other prefrontal cortex regions. Antipsychotic drugs haloperidol and clozapine, which target monoamine receptors, as well as an mGlu2/3 agonist and an mGlu5 receptor modulator proposed to have antipsychotic efficacy, reversed the impact of NMDA hypofunction on OFC cells and on behavior. A similar pattern of normalization of OFC activity was observed when treatments were given after amphetamine. Thus, proven or putative antipsychotic drugs with different mechanisms of action similarly reduced the impact of NMDA hypofunction and dopamine hyperfunction on OFC neurons, suggesting that these neurons are a candidate target for the therapeutic effects of antipsychotic medications.

amphetamine | NMDA | dopamine | prefrontal cortex | schizophrenia

Two longstanding views about the pathophysiology of schizophrenia state that it is associated with a hyperactive dopamine system (1, 2) and a state of "hypofrontality," the latter referring to reduced activation of the dorsal prefrontal cortex (PFC) during performance of cognitive tasks with a working memory component (3, 4). The dopaminergic hyperactivity is linked to the so-called positive symptoms, which include hallucinations and delusions, whereas hypofrontality is thought to subserve the cognitive deficits associated with schizophrenia. Several recent findings, however, have questioned these prevailing notions. Specifically, the principal finding that supports a role for dopamine hyperactivity in schizophrenia has been that all antipsychotic drugs, which are effective in treating these symptoms, inhibit the dopamine D2 receptors. A recent report that an agonist of the metabotropic glutamate 2/3 (mGlu2/3) receptor has comparable efficacy to the antipsychotic drug olanzapine in treating positive and negative symptoms of schizophrenia (5) suggests that blocking dopamine receptors is not necessary for antipsychotic efficacy. The notion of a cortical hypoactivity that is limited to dorsal PFC regions, in particular dorsolateral PFC, has also been questioned by recent functional imaging, structural, and postmortem studies demonstrating hyperactivity as well as hypoactivity in ventral regions of the PFC regions, in particular the orbitofrontal cortex (OFC). Unlike the deficits associated with dorsal regions of the PFC, which are selectively cognitive, abnormalities associated with the OFC correlate with positive (6) and affective (7–10) symptoms, as well as cognitive deficits of schizophrenia (11, 12).

The finding that compounds with different mechanisms of action—that is, agonists of mGlu2/3 receptors and antagonists of dopamine D2 receptors—have similar efficacies in treating positive and negative symptoms of schizophrenia suggests that these compounds may share a common cellular target. We hypothesized that OFC neurons may be such a target. OFC neurons are involved in sensory integration, feedback processing, and extradimensional set shifting, allowing this region to play a key role in goal- and context-appropriate behavioral planning (13–15). We reasoned that these are the functions that are fundamentally disrupted in schizophrenia, leading to aberrant perception and deficient affective processing, which manifest as positive and negative symptoms of the disease, respectively. If OFC is a key region in the pathogenesis of schizophrenia, then it would be expected that its activity is disrupted in animal models of the disease and that it is a target for antipsychotic agents. We first characterized the impact of NMDA hypofunction, a model with predictive, construct, and face validity for some aspects of schizophrenia (16), on the spontaneous activity of OFC neurons in behaving animals. Treatment with an NMDA antagonist increased the spontaneous activity of most pyramidal cells at the same time that it inhibited the activity of putative inhibitory GABA interneurons and increased behavioral stereotypy. We then examined the effects of pretreatment with haloperidol, a D2 antagonist and a typical antipsychotic drug; clozapine, an atypical antipsychotic drug with a wide range of affinity on dopamine and serotonin receptors; LY354740, a selective mGlu2/3 agonist (5, 17); and CDPPB, a novel mGlu5 receptor-positive allosteric modulator that has been proposed to have antipsychotic efficacy (18, 19), on NMDA antagonist-induced disrupted OFC neuronal activity and behavior. To further establish the clinical utility of our results, we also examined whether normalization of activity by antipsychotic drugs is observed after the induction of NMDA receptor hypofunction. Finally, we noted that in the OFC (but not in the medial PFC) NMDA receptor antagonists disrupt neuronal activity similarly to another psychotomimetic compound and dopamine releaser, amphetamine (20). Therefore, we also investigated whether the effects of antipsychotic drugs in normalizing OFC neuronal activity generalize to amphetamine-induced OFC hyperactivity.

Results

Differential Response of Regular Firing and Fast Firing Neurons to NMDA Receptor Blockade. Single units were classified as either regular firing (RF; putative pyramidal neurons, $n = 582$) or fast

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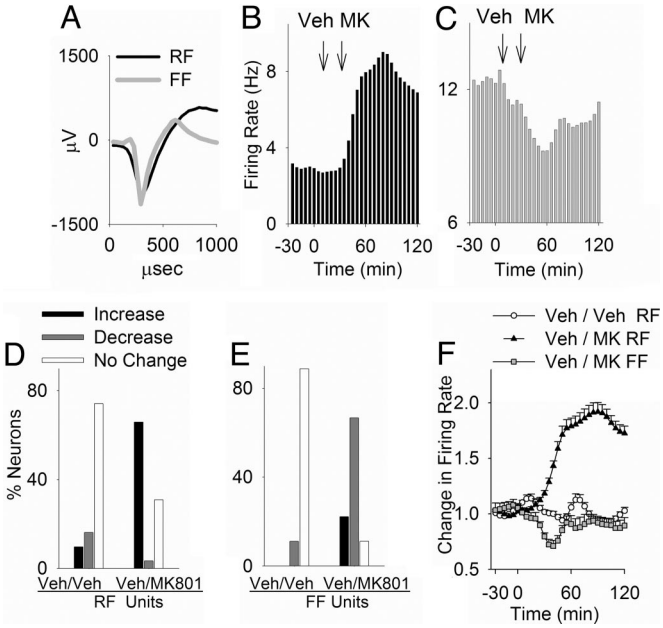

Fig. 1. Distinct effects of NMDA receptor inhibition on RF and FF neurons in the OFC. (*A*) Average waveforms of an RF and an FF unit are compared. The waveforms remained stable during the session. (*B* and *C*) Representative firing rate histograms of individual OFC RF (*B*) and FF (*C*) units in response to MK801 (0.1 mg/kg i.p.). Note the sustained firing increase in RF units and sustained firing decrease in FF units. Systemic injections were made at 0 and 20 min. (*D* and *E*) Distribution of significant firing rate responses (increase, decrease, no change) among RF and FF units. (*F*) Comparison of average (±SEM) firing rates of all RF and FF units in response to MK801. Average response of all RF units in vehicle/vehicle (Veh/Veh) group is also shown.

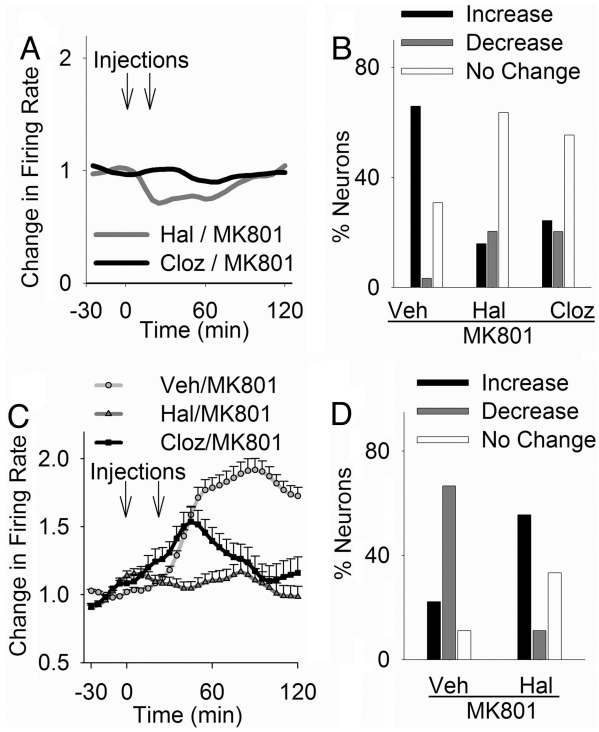

Fig. 2. Antipsychotic agents reversed the MK801 effects on OFC RF units. (*A*) Superimposed firing rate histograms of individual neurons pretreated with haloperidol (Hal; 0.1 mg/kg i.p.) or clozapine (Cloz; 10 mg/kg i.p.), followed 20 min later by MK801. (*B*) Both haloperidol and clozapine significantly decreased the proportion of neurons that showed an increase in firing response after MK801 (shown for comparison). (*C*) The average (±SEM) firing rates of all RF units pretreated with haloperidol or clozapine compared with the vehicle pretreated group (Veh). Both antipsychotic drugs inhibited the sustained excitatory effects of MK801. (*D*) Distribution of FF unit responses to MK801. Haloperidol significantly decreased the proportion of inhibitory responses to MK801 and increased the excitatory responses within this subset. Note that the number of recorded units in the clozapine group ($n = 3$) was insufficient for analysis.

firing (FF; putative interneurons, $n = 41$) units. The fast-firing units were characterized by faster firing rate (baseline, 13.2 Hz vs. 3.8 Hz), narrower spike waveforms (peak-to-valley width, 283.6 μs vs. 587.3 μs; Fig. 1*A*), and high-frequency components in their interspike interval and autocorrelation histograms. Systemic treatment with the NMDA antagonist MK801 caused sustained firing changes that were primarily excitatory in RF neurons and inhibitory in FF units. This is consistent with findings in the hippocampus (21) and medial PFC regions (22, 23). Examples are depicted in Fig. 1 *B* and *C*. More than 80% of RF units displayed a sustained excitatory response ($\chi^2 = 84.5$, $P < 0.001$ vs. vehicle/vehicle group; Fig. 1*D*), whereas more than 70% of FF units showed sustained inhibition ($\chi^2 = 11.01$, $P < 0.005$; Fig. 1*E*) in response to MK801 treatment. Comparison of the temporal profile of average firing rates of all RF units between vehicle/MK801 and vehicle/vehicle groups (ANOVA with time as repeated measure; Fig. 1*F*) revealed a significant effect for both groups ($F_{(1,224)} = 121.63$, $P < 0.001$) and time ($F_{(29,6496)} = 54.21$, $P < 0.001$) as well as for group × time interaction ($F_{(29,6496)} = 69.67$, $P < 0.001$). Within the vehicle/MK801 group, FF units showed a significantly different time course from RF units ($P < 0.001$).

Typical and Atypical Antipsychotic Drugs Reverse the Effects of NMDA Antagonist Blockade on OFC Neurons. Systemic pretreatment with haloperidol or clozapine decreased the sustained excitatory effect of MK801 on OFC RF units (Fig. 2*A*). This was apparent in both the relative number of responses (haloperidol, $\chi^2 = 58.99$, $P < 0.001$; clozapine, $\chi^2 = 41.39$, $P < 0.001$) (Fig. 2*B*) and changes in average firing rate (ANOVA, vehicle vs. haloperidol pretreatment: group, $F_{(1,188)} = 46.42$, $P < 0.001$; time, $F_{(29,5452)} = 41.63$, $P < 0.001$; group × time interaction, $F_{(29,5452)} = 33.09$, $P < 0.001$; vehicle vs. clozapine pretreatment: group, $F_{(1,182)} = 14.08$, $P < 0.05$; time, $F_{(29,5278)} = 40.06$, $P < 0.001$; group × time interaction, $F_{(29,5278)} = 21.42$, $P < 0.001$) (Fig. 2*C*).

We recorded from a small subset of FF units during these treatments [see supporting information (SI) Fig. S1 for examples of individual FF neurons]. In the haloperidol-treated group, this number was sufficient ($n = 9$) to perform statistical analysis. Haloperidol reversed the inhibitory response of FF units to MK801 ($\chi^2 = 5.85$, $P < 0.05$; Fig. 2*D*). In the clozapine group, only 3 FF units were recorded, which showed a similar response pattern.

Candidate Antipsychotic Drugs Reverse the Effects of NMDA Antagonist Blockade on OFC Neurons. Next, we examined the effects of 2 novel antipsychotic candidates with no known affinity for dopamine or other monoamine receptors but with affinities for distinct subtypes of metabotropic glutamate receptors on MK801-induced activation of OFC neurons. These included the mGlu2/3 receptor agonist LY354740 and the mGlu5 receptor-positive allosteric modulator CDPPB. Both compounds significantly reduced the excitatory response of RF units to MK801 (Fig. 3*A*, individual neuronal responses; Fig. 3*B*, LY354740, $\chi^2 = 62.26$, $P < 0.001$, and CDPPB, $\chi^2 = 86.35$, $P < 0.001$). Notably, LY354740 caused sustained inhibition in a subset of units. This effect was also reflected in the population response in this group (vs. vehicle: group, $F_{(1,160)} = 98.39$, $P < 0.001$; time, $F_{(29,4640)} = 23.17$, $P < 0.001$; group × time interaction, $F_{(29,4640)} = 35.13$, $P <$

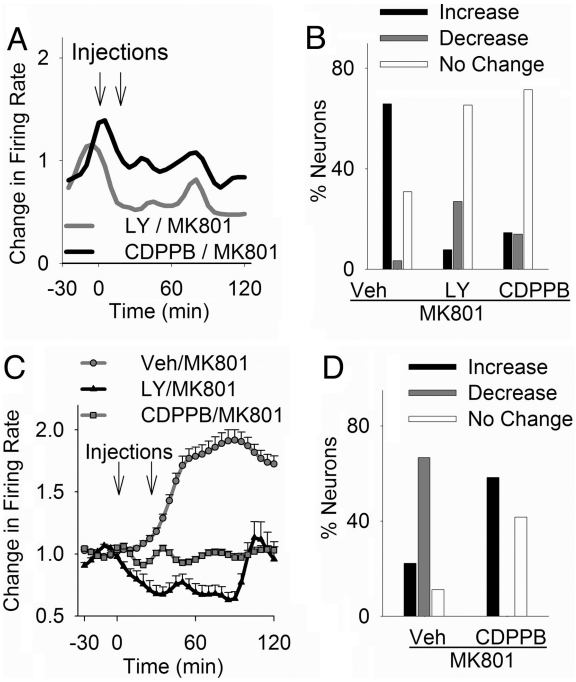

Fig. 3. Metabotropic glutamate receptor modulators blocked MK801 effects on OFC RF units. (*A*) Superimposed firing rate histograms of individual neurons pretreated with LY354740 (LY; 10 mg/kg i.p.) or CDPPB (10 mg/kg i.p.), followed 20 min later by MK801. (*B* and *C*) Both LY354740 and CDPPB inhibited the excitatory influence of MK801 on OFC units. LY354740 but not CDPPB also caused lasting inhibitory responses in average firing rate. All conventions are as in Fig. 2. (*D*) Distribution of FF unit responses to MK801. Similarly to haloperidol, CDPPB decreased the proportion of inhibitory responses to MK801. Veh indicates vehicle.

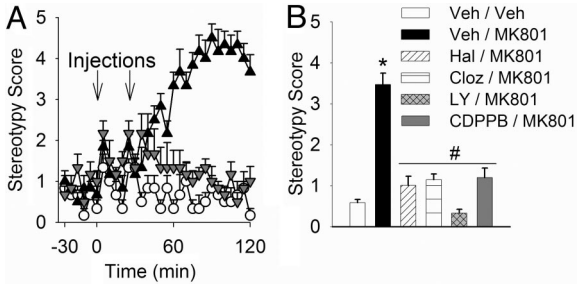

Fig. 4. Behavioral stereotypy. (*A*) Average stereotypy scores during recording sessions (5-min bins) are shown for vehicle/vehicle (Veh/Veh), vehicle/MK801, and CDPPB/MK801 groups. The results for other groups were not demonstrated for clarity. (*B*) Average stereotypy scores for the post-MK801 period (minutes 10–120) are compared between all groups. Both antipsychotic agents as well as LY354740 (LY) and CDPPB blocked MK801-induced stereotypy. Hal indicates haloperidol; Cloz, clozapine. *, $P < 0.05$ compared with Veh/Veh; #, $P < 0.05$ compared with Veh/MK801.

0.001) (Fig. 3*C*). CDPPB blocked the excitatory effect of MK801 over time (group, $F_{(1,262)} = 150.4$, $P < 0.001$; time, $F_{(29,7598)} = 76.64$, $P < 0.001$; group × time interaction, $F_{(29,7598)} = 80.14$, $P < 0.001$) without causing inhibition. A relatively small subset of units in the CDPPB-treated group were characterized as FF units (see example in Fig. S1). In this group, CDPPB produced an effect similar to haloperidol, reversing the inhibitory effects of NMDA receptor blockade on this subset of neurons ($\chi^2 = 11.59$, $P < 0.005$; Fig. 3*D*).

To better simulate conditions in which antipsychotic medications are administered in the context of already present NMDA receptor hypofunction, we also examined the impact of posttreatment with drugs after MK801 administration. Again, the excitatory effect of MK801 was reversed by posttreatment with haloperidol, LY354740, and CDPPB, an effect that was reflected both in the proportion of cells with excitatory responses to MK801 and in the average firing rates of neurons in each treatment group (Fig. S2).

Reversal of NMDA Antagonist-Induced Behavioral Stereotypy by Established and Candidate Antipsychotic Drugs. During the recording sessions, behavioral stereotypy was measured as an index of behavioral activation by MK801. The temporal profile and average post-MK801 stereotypy scores are shown on Fig. 4 *A* and *B*, respectively. Haloperidol, clozapine, LY354740, and CDPPB similarly reversed the MK801 stereotypy (ANOVA, $F_{(5,25)} = 32.45$, $P < 0.001$; post hoc analysis of each group vs. vehicle, $P < 0.05$).

Disruption of OFC Neuronal Activity by Amphetamine Is Reversed by Antipsychotic Drugs. The psychotomimetic compound amphetamine produces an excitatory influence on OFC RF neurons similar to that observed here with MK801, which is in contrast to the effect of amphetamine in the medial PFC (20). We reasoned that if normalization of hyperactive OFC neurons is a key mechanism of action for antipsychotic drugs, then it should generalize to amphetamine-induced OFC neuron hyperactivation. Thus, we treated animals with a dose of amphetamine that produced levels of activation of OFC RF neurons similar to those of MK801 and then compared the effects of posttreatment with vehicle, a classic antipsychotic drug (haloperidol), and a candidate compound (CDPPB). Amphetamine caused an excitatory response in the majority of OFC RF units (example in Fig. 5*A*). Posttreatment with either haloperidol (0.1 mg/kg) or CDPPB (10 mg/kg) reversed this excitatory effect (examples in Fig. 5*A*). Among the population of RF units, the predominantly excitatory effect of amphetamine was reversed by both haloperidol ($\chi^2 = 43.92$, $P < 0.001$; Fig. 5*B*) and CDPPB ($\chi^2 = 28.68$, $P < 0.001$). Comparing the population responses among RF units in each treatment group, 2-way ANOVA revealed significant effects for both posttreatment groups compared with vehicle (haloperidol: group, $F_{(1,102)} = 32.38$, $P < 0.001$; time, $F_{(29,2958)} = 24.39$, $P < 0.001$; group × time interaction, $F_{(29,2958)} = 14.28$, $P < 0.001$; CDPPB: group, $F_{(1,108)} = 5.49$, $P < 0.05$; time, $F_{(29,3132)} = 58.25$, $P < 0.001$; group × time interaction, $F_{(29,3132)} = 22.01$, $P < 0.001$) (Fig. 5*C*). This reversal of neuronal activity was also associated with reversal of amphetamine-induced stereotypy by both haloperidol and CDPPB ($P < 0.01$; Fig. 5*D*).

Discussion

Use-dependent blockade of NMDA receptors, which provides a pharmacological model of schizophrenia (16, 24), profoundly increased the spontaneous activity of putative pyramidal cells in the OFC, and at the same time inhibited the activity of putative inhibitory GABAergic interneurons and increased behavioral stereotypy. The pattern of activation mimicked the effect of amphetamine on OFC pyramidal neurons (20). Amphetamine is a dopamine releaser and psychotomimetic drug (25) that is commonly used to model a hyperdopaminergic state in schizophrenia (16). The similar effects of 2 psychotomimetic compounds with different mechanisms of action on OFC neurons are consistent with clinical studies demonstrating hyperactivity of OFC regions in individuals with schizophrenia (26–28). Treatment with 4 compounds with distinct mechanisms of action that are either well-established antipsychotic compounds or novel candidates for treatment of schizophrenia ameliorated the impact of NMDA hypofunction on OFC neurons and on behavior. These compounds included (*i*) haloperidol, a D2 antagonist and a typical antipsychotic drug; (*ii*) clozapine, an atypical antipsy-

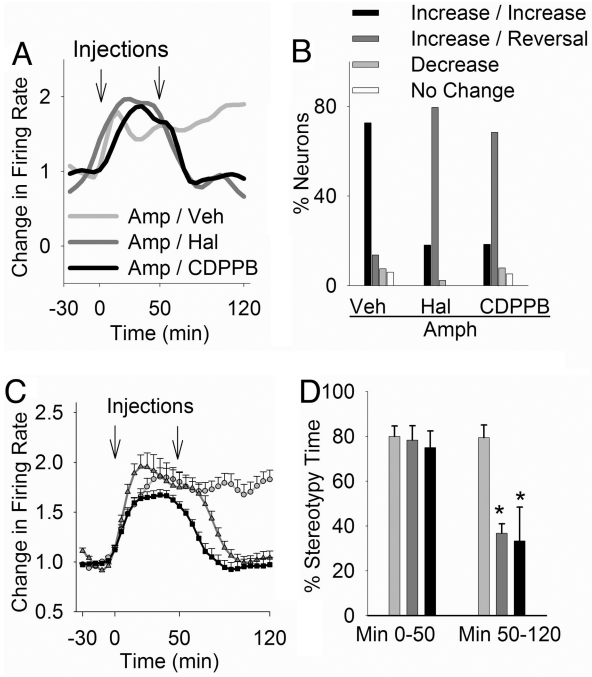

Fig. 5. Response of OFC RF units to amphetamine (Amp). (*A*) Representative firing rate histograms of individual OFC RF units treated with amphetamine followed 50 min later by vehicle (Veh), haloperidol (Hal; 0.1 mg/kg i.p.), or CDPPB (10 mg/kg i.p.). All 3 units showed an excitatory response to MK801 that was sustained in the vehicle posttreated unit but was reversed in the haloperidol or CDPPB posttreated units. (*B*) Amphetamine (Amph) caused an excitatory response in the majority of OFC units. This response was reversed in the majority of neurons in the haloperidol and CDPPB groups. (*C*) The mean (±SEM) firing rates of all RF units in each group. (*D*) Stereotypy score in the amphetamine group. Stereotypy was scored based on the percentage of time spent on stereotypical behavior (rearing, up and down sniffing, turning, and ambulating) during 30-s windows assessed every 5 min. Average scores for minutes 0–50 (post amphetamine) and minutes 50–120 (post haloperidol or post-CDPPB) are shown separately. In all 3 groups, amphetamine caused significant stereotypy. This effect was reversed by haloperidol and CDPPB posttreatments. *C* and *D* are color coded as in *A* and *B*.

chotic drug with a wide range of affinity at dopamine and serotonin receptors; (*iii*) LY354740, a selective mGlu2/3 agonist with no affinity for dopamine or serotonin receptors that has been suggested recently to have antipsychotic efficacy (5); and (*iv*) CDPPB, a novel mGlu5 receptor-positive allosteric modulator that has also been proposed as a potential antipsychotic agent (18, 19). In a similar way, haloperidol and CDPPB reversed amphetamine-induced hyperactivation of OFC neurons. Together, these findings suggest that normalization of OFC neuronal activity, whether caused by NMDA receptor hypofunction or excess dopamine neurotransmission, may be a common target for different classes of antipsychotic drugs. Thus, determining the ability of compounds to normalize OFC activity may provide an evaluation of antipsychotic potential.

OFC Dysfunction and Schizophrenia. The OFC receives extensive input from sensory, limbic, and basal ganglia regions and plays a critical role in sensory integration and feedback processing (29). Disruption in OFC function may lead to inappropriate integration of relevant sensory stimuli and previously learned associations, and failure in suppression of irrelevant stimuli and associations (14, 30, 31). This mode of disruption may be a critical component of positive and negative symptoms as well as cognitive deficits of schizophrenia. Multidisciplinary lines of evidence have, in fact, reported various OFC abnormalities in schizophrenia that are associated with different symptoms of the disease (6–12, 32, 33). For example, MRI studies have reported volume deficits in association with thought disorder (6) and severity of negative symptoms (9, 34, 35). Longitudinal MRI studies in individuals at risk to develop schizophrenia have shown reduction in OFC gray matter in those who develop psychosis (36). These latter findings, together with another study that reported OFC volume deficits in drug-naive patients (37), strongly suggest that OFC anomalies are components of the disease process and not a consequence of chronic antipsychotic drug treatment. Accordingly, individuals with schizophrenia show significant performance deficits in cognitive paradigms, such as a probabilistic reversal learning task and the Iowa Gambling Task, which involve feedback processing and which are classically used to assess OFC function (12, 38, 39). In related functional imaging studies, hyperactivation of OFC is reported in patients during performance of cognitive tasks that generally occur concomitantly with hypoactivation of dorsal PFC regions (26, 27). Finally, in one of the few studies reporting regional metabolic activity in actively hallucinating individuals with schizophrenia, significant hyperactivity of OFC was reported (28). This is consistent with our findings that in 2 animal models of schizophrenia, the NMDA hypofunction model (the present study) and the amphetamine model (20), there is general overactivation of OFC neurons.

Although the major focus of postmortem work in schizophrenia has been on dorsal regions of the PFC, a few interesting findings have been reported in the OFC. These include increases in glial fibrillary acidic protein, changes in the density of NMDA and kainate receptor subunits (40), and dramatic decreases in the subtypes of dopamine receptors localized within the OFC that are not observed in other PFC regions or the striatum (41). Significant postmortem changes in the distribution of Disrupted-In-Schizophrenia (DISC1) protein also seem to be selective to OFC regions (42). *DISC1* has been identified recently as a susceptibility gene for schizophrenia (43, 44), and a DISC1 polymorphism has been correlated with severity of positive symptoms (45).

Different Pharmacological Agents with Antipsychotic Efficacies Similarly Normalize OFC Neuronal Firing. Causes of schizophrenia remain elusive, but recent genetic studies suggest that multiple mutations (46), including rare mutations (47), may contribute to the pathophysiology of the disease. This suggests that changes in the function of a variety of molecules may lead to disruption in the function of a common group of neurons and cellular networks. Thus, although the focus of research for treating the disorder has been on normalizing the function of a single protein (such as the D2 receptor), it is reasonable to suggest that this focus should be expanded to include normalizing the function of groups of neurons and their associated networks. Until recently, the dopamine D2 receptor was the only established target for treatment of schizophrenia. New findings that an mGlu2/3 receptor agonist that is devoid of activity at the D2 receptor has a profile of antipsychotic efficacy similar to that of an approved antipsychotic drug (5) provides an important tool for discovering neuronal networks that serve as common targets for established and novel antipsychotic agents and may be critical for treatment of schizophrenia. Although cortical dysfunction has long been acknowledged as the main site of pathology in schizophrenia, this research has been focused mostly on the dorsal and medial regions of the PFC (48, 49). Neurons in these regions are likely to play a role in aspects of cognitive deficits associated with the disease; however, it is unlikely that they account for the whole spectrum of the therapeutic properties of antipsychotic drugs; functional alterations in these regions are generally not associated with noncognitive symptoms of schizophrenia, and antipsychotic drugs do not consistently improve the cognitive deficits

of this disorder (36, 46, 49). Here, we postulated that the OFC is a key cortical component of the distributed networks targeted by antipsychotic drugs because functional changes in this region have been observed during active psychosis (28) and are associated with the negative symptoms and cognitive deficits of schizophrenia (see above). We found that NMDA antagonists, as with another psychotomimetic compound, amphetamine, similarly increased the activity of the majority of cortical pyramidal neurons. Although a similar pattern of response to NMDA antagonists (50) and antipsychotic drugs has been reported in the medial and dorsal regions of the PFC (51, 52), it is important to emphasize that amphetamine leads to a primarily inhibitory response in other cortical regions (20). Thus, the present finding is in line with OFC activation reported during active psychosis (28) and suggests that both of these pharmacological treatments provide comparable dynamic models of the OFC hyperactivity that may be present in schizophrenia. That compounds with antipsychotic efficacy (or potential) with very different mechanisms of action, and different effects on OFC neuronal activity when administered alone, similarly reduce the impact of NMDA receptor hypofunction and amphetamine on OFC neurons further suggests that normalizing the disrupted balance of excitatory and inhibitory activity in OFC may be critical for treatment of schizophrenia.

Further work is required to examine the molecular mechanisms by which different classes of compounds can have similar downstream effects on OFC pyramidal and GABAergic neurons. Although the present study is limited to reporting a dynamic cellular phenomenon, it demonstrates that NMDA receptor hypofunction and dopamine hyperfunction, both of which are suspected to occur in schizophrenia, leads to a similar disruption in the activity of OFC neurons. Furthermore, compounds that work on distinct proteins and receptor classes can have comparable effects in reversing these disruptions. These findings support the notion that the key to understanding the pathophysiology of schizophrenia and the design of better treatments is to identify the common group of neurons, and the functional networks influenced by these neurons, that are similarly affected by the diverse, and often rare mutations, that are associated with this brain disorder.

Materials and Methods

Detailed methods are included as *SI Experimental Procedures*. Sprague–Dawley male adult rats were used. All experimental protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (53). Details of surgery, recording, and isolation of single units have been published previously (20, 50).

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