Activation of Metabotropic Glutamate 2/3 Receptors Reverses the Effects of NMDA Receptor Hypofunction on Prefrontal Cortex Unit Activity in Awake Rats

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Homayoun, Houman, Mark E. Jackson, and Bita Moghaddam. Activation of metabotropic glutamate 2/3 receptors reverses the effects of NMDA receptor hypofunction on prefrontal cortex unit activity in awake rats. J Neurophysiol 93: 1989–2001, 2005. First published December 8, 2004; doi:10.1152/jn.00875.2004. Systemic exposure to N-methyl-D-aspartate (NMDA) receptor antagonists can lead to psychosis and prefrontal cortex (PFC)–dependent behavioral impairments. Agonists of metabotropic glutamate 2/3 (mGlu2/3) receptors ameliorate the adverse behavioral effects of NMDA antagonists in humans and laboratory animals, and are being considered as a novel treatment for some symptoms of schizophrenia. Despite the compelling behavioral data, the cellular mechanisms by which potentiation of mGlu2/3 receptor function attenuates the effects of NMDA receptor hypofunction remain unclear. In freely moving rats, we recorded the response of medial PFC (prelimbic) single units to treatment with the NMDA antagonist MK801 and assessed the dose-dependent effects of pre- or posttreatment with the mGlu2/3 receptor agonist LY354740 on this response. NMDA receptor antagonist–induced behavioral stereotypy was measured during recording because it may relate to the psychotomimetic properties of this treatment and is dependent on the functional integrity of the PFC. In most PFC neurons, systemic administration of MK801 increased the spontaneous firing rate, decreased the variability of spike trains, and disrupted patterns of spontaneous bursts. Given alone, LY354740 (1, 3, and 10 mg/kg) decreased spontaneous activity of PFC neurons at the highest dose. Pre- or posttreatment with LY354740 blocked MK801-induced changes on firing rate, burst activity, and variability of spike activity. These physiological changes coincided with a reduction in MK801-induced behavioral stereotypy by LY354740. These data indicate that activation of mGlu2/3 receptors reduces the disruptive effects of NMDA receptor hypofunction on the spontaneous spike activity and bursting of PFC neurons. This mechanism may provide a physiological basis for reversal of NMDA antagonist–induced behaviors by mGlu2/3 agonists.

INTRODUCTION

Psychopharmacological studies in humans (Krystal et al. 1994; Lahti et al. 1995; Malhotra et al. 1996; Newcomer et al. 1999) and behavioral studies in laboratory animals (Buffalo et al. 1994; Carlsson and Carlsson 1990; Javitt et al. 1996; Koek et al. 1988; Verma and Moghaddam 1996) indicate that treatment with N-methyl-D-aspartate (NMDA) receptor antagonists produces a host of behavioral abnormalities that resemble the positive symptoms, the negative symptoms, and the cognitive deficits associated with schizophrenia. The NMDA antagonist treatment is thus considered to be a model with face validity for the symptoms of schizophrenia (Geyer and Moghaddam 2002). Accordingly, pharmacological interventions that normalize the adverse behavioral effects of NMDA receptor antagonist treatment are under evaluation as potential antischizophrenia therapies (Coyle et al. 2002; Javitt et al. 2001; Krystal et al. 2003; Moghaddam 2003; Nilsson et al. 1997). Among such targets are activators of metabotropic glutamate 2/3 (mGlu2/3) receptors. Compounds of this class reduce behavioral stereotypy, hyperlocomotion, and working memory deficits produced by NMDA antagonists, such as phencyclidine, in rodents (Carlson et al. 1999; Moghaddam and Adams 1998). In addition, recent human studies indicate that mGlu2/3 agonists ameliorate the cognitive effects of another NMDA receptor antagonist, ketamine, in human volunteers (Krystal et al. 2004). However, although mGlu2/3 receptors are thought to have autoregulatory effects on presynaptic glutamate release (Kilbride et al. 1998; Marek et al. 2000; Schoepp et al. 1999), the cellular mechanisms by which activation of these receptors reverses the effects of NMDA antagonists in behaving animals remain to be elucidated.

Consistent with the plethora of findings that implicate prefrontal cortex (PFC) dysfunction in the pathophysiology of schizophrenia (Knable and Weinberger 1997), several lines of evidence suggest that PFC plays a critical role in the behavioral effects of NMDA antagonists. First, inactivation of PFC in rats inhibits the stereotypy and hyperlocomotion associated with NMDA antagonists (Jentsch et al. 1998; Takahata and Moghaddam 2003). These behaviors in the rodent are thought to be relevant to symptoms of schizophrenia because stereotyped tendencies are a critical component of schizophrenic symptomatology (Carlsson et al. 1993; Geyer and Moghaddam 2002; Tracy et al. 1996). Second, human volunteers treated with NMDA receptor antagonists show impairments in the performance of PFC-dependent tasks, such as the n-back and Wisconsin Card Sort tasks (Adler et al. 1998; Krystal et al. 1994). A similar impairment in PFC-dependent tasks that assess working memory or set-shifting behavior has been observed in rats in response to NMDA receptor antagonists (Schmidt 1994; Stefani et al. 2003; Verma and Moghaddam 1996). Finally, human imaging studies have shown that NMDA antagonists alter PFC metabolic activity (Breier et al. 1997; Duncan et al. 2000). Recently, we characterized the effects of treatment with the NMDA receptor antagonist MK801 on spontaneous single-unit activity of PFC neurons in...
awake rats (Jackson et al. 2004). MK801 produced a profound disruptive effect on the spontaneous firing and bursting of the majority of PFC neurons, increasing the tonic firing rate while decreasing burst activity. Assuming that the functional state of PFC neurons plays a critical role in the cognitive impairing and psychotomimetic effects of NMDA receptor antagonists (Takahata and Moghaddam 2003), we sought to test the hypothesis that activation of mGlu2/3 receptors, at doses that reduce the expression of these behaviors, attenuates the NMDA antagonist–induced disruption of neuronal activity in the PFC. Thus we examined the effects of pre- or posttreatment with the mGlu2/3 receptor agonist LY354740 on MK801-induced alterations in PFC neuronal firing in freely moving rats.

M E T H O D S

S u b j e c t s

Fourteen adult male Sprague–Dawley rats weighing 340–420 g were used in this study. Animals were individually housed on a 12-h light/12-h dark cycle. Experiments were performed during the light phase. All experimental protocols were approved by the 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.

E lectrophysiological recording

Chronically implanted microelectrode arrays (NB Labs, Denison, TX) were used for all single-unit recordings. Arrays consisted of eight 50-μm-diameter Teflon-insulated, stainless steel wires arranged in a 2 × 4 pattern measuring about 0.25 × 0.7 mm. Electrode arrays were implanted under halothane anesthesia in the medial PFC (target coordinate for the center of array at AP +3.0, ML 0.7, and DV –3.5; Fig. 1A), according to the atlas of Paxinos and Watson (1986). Reference electrodes were placed posterior to the recording electrodes. Ground wires were connected to stainless steel skull screws. The electrode array was secured onto the cranium with dental cement using additional scull screws as anchors. Animals were allowed 1 wk to recover from surgery before the start of experiments. All recordings were performed in the animals’ home cage (clear polycarbonate 44 × 22 × 42 cm) with a modified open top extending an additional 42 cm above the cage. Animals were connected to a FET headstage (NB Labs) by means of lightweight cabling that passed through a 24-channel commutator (NB Labs), which allowed the animal to move unrestricted during recording. Extracellular unit activity was recorded using multiple-channel amplifiers with 500 × gain and 220-Hz to 5.9-kHz band-pass filters (Plexon, Dallas, TX). The amplified signal from each electrode was digitized (30-kHz sampling rate) and continuous data files were saved on a PC computer hard disk for off-line spike sorting. To reduce the movement artifact, the signal from a reference electrode was subtracted from each individual wire’s signal. Spike sorting was performed with Off-Line Sorter software (Plexon) using a combination of automatic and manual sorting techniques (for review, see Lewicki 1998). The first 3 principal components of all waveforms recorded from each electrode were depicted in 3-dimensional space. Automatic clustering techniques (k-means clustering and valley seeking methods) were used to produce an initial separation of waveforms into individual units. Each cluster was then checked manually to ensure that the cluster boundaries were well separated and

![Image](image-url)

**FIG. 1.** A: placement of electrodes. Arrays were 0.7 mm long and 0.25 mm wide and were placed lengthwise along the anterior–posterior axis. Numbers under each coronal section indicate the distance anterior to bregma (Paxinos and Watson 1986). B: sample normalized rate histograms of medial prefrontal cortex (mPFC) neurons recorded during vehicle/vehicle injection (indicated by arrows). Bins are 300 s and y-axis shows firing rate as spikes/s. Mean and 99% confidence intervals (dashed lines) of baseline firing rate were calculated and then used to detect significant long-term changes in firing rate (A). Two consecutive bins exceeding the confidence intervals were considered a significant response. C and D: samples of the spike trains of 2 neurons in vehicle/vehicle group during the baseline period (C) and after second injection (D) with burst periods. Bursts (indicated by horizontal lines) were detected by Poisson surprise method of Legendy and Salcman (1985; see METHODS). Burst detection threshold used in this study (surprise value = 5) finds the periods of burst firing that have a probability of occurrence ≥150 times greater than what may be expected in a Poisson Spike train with the same firing rate. Neurons depicted here did not show a change in mean firing rate but altered their bursting activity.

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waveform shapes were consistent with action potentials. The waveforms in each selected cluster were averaged to produce computer-generated templates that were then used to match spikes recorded during each single session. Obvious artifacts were removed and the stability of clusters throughout the experiment was confirmed by plotting the first principal component versus the timestamp for each waveform. The resultant clusters were chosen as single units only if autocorrelograms and interspike interval (ISI) histograms indicated that there were no significant errors in sorting arising from noise or similar waveforms. An absolute refractory period of \( \geq 1.1 \text{ ms} \) was used to select single units. Typically, 2 to 3 neurons were isolated from each electrode. Although recording with chronically implanted electrodes can lead to stable recordings across sessions, the shape of the clusters and waveforms may change from session to session. In this study, each animal received a given treatment only once and waveform templates were not matched across sessions.

Based on previously reported criteria (Jung et al. 1998; McCormick et al. 1985) on firing rate (FR) and autocorrelogram statistics, neurons were divided into regular spiking (FR < 10, sporadic firing pattern in autocorrelogram, putative pyramidal neurons) and fast spiking (FR > 10, tonic firing pattern, putative interneurons) units. However, because very few units met the criteria of fast-spiking neurons (total number 14 with 0–2 units per group), reliable statistical analysis could not be performed for this subgroup. Thus all reported results are based on regular-spiking single units.

**Experimental procedures**

After the surgical recovery period, animals were acclimated to the recording environment by daily transportation from the housing facility to the recording room where they were hooked up to the recording apparatus for 2 h. After 1 wk of acclimation, animals received 2–4 sessions of drug administration with 1-wk intersession intervals. Each treatment was administered to \( \geq 4 \) animals and each animal received a given treatment only once with a pseudo-random order of administration. Each session consisted of a 1-h habituation, 30-min baseline recording before the first injection, and \( \leq 3 \)-h recording after the last injection.

The NMDA antagonist MK801 (Sigma-RBI, St. Louis, MO) and the mGlu2/3 agonist LY354740 (gift from E. Lilly) were both dissolved in saline and injected intraperitoneally (ip). In pretreatment experiments, LY354740 (1, 3, or 10 mg/kg) or vehicle was administered 20 min before MK801 (0.1 mg/kg) or vehicle (saline). In posttreatment experiments, the mGlu2/3 agonist was injected 50 min after to allow MK801 to reach its maximal effect (Table 1). The mGlu2/3 agonist LY354740 (10 mg/kg) or vehicle was administered 50 min after MK801 to reach its maximal effect (Table 1). The dosage and time interval for MK801 and LY354740 injections were chosen based on previous electrophysiological and behavioral findings (Cartmell et al. 1999; Jackson et al. 2004; Moghaddam and Adams 1998).

**Data analysis**

Electrophysiological data were imported into NeuroExplorer (Plexon) for analysis. Firing rate statistics were calculated using firing rate histograms with 5-min bins. For each isolated unit, the mean spontaneous firing rate and 99% confidence intervals were calculated for the 30-min baseline period. The firing rate for each neuron was normalized relative to baseline (Fig. 1B). Changes in firing rate attributed to MK801 and its modulation by LY354740 were measured by comparing each normalized rate histogram bin after the second injection to the normalized baseline firing rate. A significant change in firing rate was defined as 2 consecutive 5-min bins exceeding the 99% confidence intervals of the baseline (combined probability of \( P < 0.0001 \)). Termination of a significant response also required 2 consecutive 5-min bins within the 99% confidence intervals of the baseline. This criterion was chosen because we were interested in the long-lasting effects of different treatments on the tonic firing of PFC neurons. Neuronal responses were divided into one of 3 response types depending on whether they showed significant increase (type 1), decrease (type 2), or no change (type 3) in spontaneous firing rate. To avoid the transient effects of the injection, changes in firing rate that occurred within 10 min after the second injection were not analyzed. Neurons were grouped by response types for further analysis.

The Pearson chi-square (\( \chi^2 \)) test was used to determine significant differences in the proportion of response types between groups. Normalized rate histograms for each response type were compared across different groups using one-way repeated-measures ANOVA with time as the repeated measure. Wherever a significant effect was observed, further analysis between 2 groups was carried out using one-way ANOVA with time as the repeated measure followed by Bonferroni post hoc test. In addition, the average magnitude and mean duration of increase and decrease responses were calculated and compared across groups using a one-way ANOVA with Bonferroni post hoc test.

Neurons typically generate action potentials in patterns that range between 2 modes: single spiking and high-frequency phasic bursting. Single-spike patterns may be either tonic or irregular, whereas phasic-bursting patterns consist of a sudden onset of a cluster of action potentials emitted at relatively higher frequency. In the awake animal, these modes are not fixed, and a single neuron often switches back and forth between the 2 modes as the neuron receives afferent input from different ensembles of neurons (Cooper 2002). In the present study, bursts and burst-related statistics were determined using the Poisson surprise method as implemented in NeuroExplorer. It should be emphasized that there are 2 commonly used methods of burst detection that are based on ISI distribution (see Boraud et al. 2002; Kaneoke and Vitek 1996). In the Poisson surprise method (Legendy and Salcman 1985), which we use here, a burst is defined as a group of spikes in which several successive ISIs do not follow a Poisson

**Table 1. Treatment schedule and mean baseline firing rate of recorded single units in different groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Injection 1, min</th>
<th>Injection 2, min</th>
<th>N</th>
<th>Baseline Firing Rate, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle (30)</td>
<td>Vehicle (50)</td>
<td>153</td>
<td>2.54 ± 0.37</td>
</tr>
<tr>
<td>2</td>
<td>LY354740 1 mg/kg (30)</td>
<td>Vehicle (50)</td>
<td>50</td>
<td>3.49 ± 0.29</td>
</tr>
<tr>
<td>3</td>
<td>LY354740 3 mg/kg (30)</td>
<td>Vehicle (50)</td>
<td>78</td>
<td>2.89 ± 0.30</td>
</tr>
<tr>
<td>4</td>
<td>LY354740 10 mg/kg (30)</td>
<td>Vehicle (50)</td>
<td>102</td>
<td>2.03 ± 0.18</td>
</tr>
<tr>
<td>5</td>
<td>Vehicle (30)</td>
<td>MK801 0.1 mg/kg (50)</td>
<td>49</td>
<td>3.28 ± 0.41</td>
</tr>
<tr>
<td>6</td>
<td>LY354740 1 mg/kg (30)</td>
<td>MK801 0.1 mg/kg (50)</td>
<td>86</td>
<td>2.75 ± 0.25</td>
</tr>
<tr>
<td>7</td>
<td>LY354740 3 mg/kg (30)</td>
<td>MK801 0.1 mg/kg (50)</td>
<td>109</td>
<td>1.98 ± 0.22</td>
</tr>
<tr>
<td>8</td>
<td>LY354740 10 mg/kg (30)</td>
<td>MK801 0.1 mg/kg (50)</td>
<td>86</td>
<td>3.28 ± 0.21</td>
</tr>
<tr>
<td>9</td>
<td>Vehicle (30)</td>
<td>Vehicle (80)</td>
<td>105</td>
<td>2.61 ± 0.31</td>
</tr>
<tr>
<td>10</td>
<td>MK801 0.1 mg/kg (30)</td>
<td>Vehicle (80)</td>
<td>89</td>
<td>2.63 ± 0.21</td>
</tr>
<tr>
<td>11</td>
<td>MK801 0.1 mg/kg (30)</td>
<td>LY354740 3 mg/kg (80)</td>
<td>110</td>
<td>3.26 ± 0.26</td>
</tr>
<tr>
<td>12</td>
<td>MK801 0.1 mg/kg (30)</td>
<td>LY354740 10 mg/kg (80)</td>
<td>133</td>
<td>2.86 ± 0.19</td>
</tr>
</tbody>
</table>

Values are mean ± SE. The timing of the injections has been indicated as minutes after the start of the recording session. N indicates the number of neurons in each group.
distribution with the same mean firing rate (Boraud et al. 2002). A second method is the histogram method, in which multimodal ISI histograms are used to differentiate the distribution of burst ISIs from other ISIs that occur in the spike train. The histogram method is better at detecting very regular and oscillatory bursting patterns, and is commonly used to detect intrinsic bursting in hippocampal and dopaminergic neurons. However, the histogram method fails to detect bursts when the spike train has markedly varying ISIs (Kaneoke and Vitek 1996). The spike trains we record from the mPFC typically have a highly irregular pattern of ISIs that precludes the use of the histogram method. Thus the definition of bursting that is of greatest interest to us—the periods in each spike train that have a higher discharge rate than do surrounding periods in the same spike train (Kaneoke and Vitek 1996)—is better suited for detection by the Poisson surprise method. This type of bursting tends to reflect afferent driven activity as well as the intrinsic properties of the cells (Boraud et al. 2002; Cooper 2002; Cooper and White 2000). For this reason it is likely that the pattern of bursts produced by any individual neuron would be highly variable as that neuron participates in the activity of different networks. Accordingly, bursts were detected by locating consecutive ISIs that were less than half the mean ISI and testing whether these ISIs would be expected if the spike train were a Poisson process. Thus this method uses the distribution of spikes across a recorded train to detect the bursting activity for each neuron (Fig. 1C).

The surprise value is defined as the negative natural logarithm of the probability that the sequence of a group of spikes in a given time interval is significantly different from what would be expected from Poisson distribution with the same mean firing rate. Therefore this analysis is not sensitive to changes in average firing rate. The Poisson surprise value provides an estimate of the level of confidence associated with the burst detection. In a preliminary analysis (data not shown), we examined the bursting data using 3 different minimum surprise values of 3, 5, and 10, which suggests that bursts are occurring about 20 ($P < 0.05$), 150 ($P < 0.007$), or 22,000 ($P < 0.00005$) times as frequent as in a Poisson Spike train with the same firing rate, respectively. Because the general pattern of changes in bursting parameters across treatment groups was consistent across these analyses, here we report the results with a surprise value of 5.

Burst parameters measured for each spike train included the number of bursts per min, the percentage of spikes that occur in bursts, the mean number of spikes per burst, and the mean and peak frequency within bursts. Comparisons of burst parameters between groups were made using one-way ANOVA with Bonferroni post hoc test. To further analyze the temporal profile of changes in bursting activity, changes in burst parameters for the LY354740 posttreatment experiments were compared across time for each neuron.

To assess the variability of firing for each spike train, we calculated the coefficient of variation (CV) of ISI by dividing the SD of the ISIs by their mean. As a global measure of variability, CV indicates how close the spike train is to an ideal Poisson spike train (CV = 1) with the assumption that the data are stationary. However, because the drugs used in the present study changed the spontaneous firing rate of most PFC neurons, a second local measure of variability, CV2, which provides a more reliable measure of intrinsic variability of spiking processes independent of gradual changes in firing rate was used (Compte et al. 2003; Holt et al. 1996). In brief, CV2 was computed by assessing the SD and mean firing for each 2 adjacent ISIs in the spike train and calculating the 2-point coefficient of variation [$CV^2 = 2 \left[ ISI_1 - ISI_2 \right]/\left[ ISI_1 + ISI_2 \right]$]. The CV2 for each pair of adjacent ISIs was then plotted against the mean of those 2 ISIs to produce a scatter plot of CV2 values for each neuron (Holt et al. 1996). We then computed the average CV2 value for each neuron during the desired time window. The effect of drug treatment on spiking variability was assessed by within-group comparisons of the mean CV and CV2 values for all neurons in each group for separate 30-min periods using one-way ANOVA with Bonferroni post hoc test.

### Behavioral stereotypy

Stereotypical behavior was rated during the electrophysiological recording every 5 min as described before (Adams and Moghaddam 1998). In brief, animals received a score of 1 for the presence of each of the following behaviors: ambulation, turning, head wagging, grooming, sniffing up or down, digging, rearing, and mouth movement or jaw tremor. For each rat different repetitive behaviors at each 5-min point were summed to determine a time course of stereotypy scores. The nonparametric Kruskal–Wallis test was used to determine significant between-group effects ($P < 0.05$). To correlate the LY354740-induced reversal of the electrophysiological and behavioral effects of MK801, we compared the stereotypy score during each 5-min bin during the entire recording session with the average firing rate during the same 5-min bin for each rat by using Pearson correlation with the Bartlett $\chi^2$ test for significance. The rater was not blind to the treatment schedule.

### Histology

Animals were anesthetized with chloral hydrate and intracardially perfused with saline followed by 10% buffered formalin. Fixed brains were cut at 200-μm intervals and sections stained with cresyl violet to probe electrode placements. Under a light microscope, tracks of recording electrodes were confirmed to be in the PFC. Recordings were made in ventral prelimbic and dorsal infralimbic cortex (Fig. 1A).

### Results

#### Single-unit recording in PFC

In the first set of experiments involving LY354740 pretreatment, a total of 713 regular spiking single units were isolated in 33 recording sessions from rats treated with LY354740 (or vehicle) 20 min before injection of either MK801 (0.1 mg/kg) or vehicle. In the second set of experiments, which involved LY354740 posttreatment, a total of 437 regular-spiking single units were isolated in 18 recording sessions from rats administered with MK801 (0.1 mg/kg or vehicle) 50 min before LY354740 or vehicle. The baseline firing rates of the neurons in different groups were comparable across different treatment groups as shown in Table 1 [one-way ANOVA, LY pretreatment experiment, $F(7,705) = 1.10$, $P > 0.05$, LY posttreatment experiment, $F(3,433) = 1.95$, $P > 0.05$]. Sample histograms of drug-induced changes in firing rate are demonstrated in Fig. 2.

LY354740 dose-dependently decreases the firing rate of PFC neurons

Overall comparison of the proportion of responses in the groups treated with LY354740 (and vehicle) showed a significant effect ($\chi^2 = 100.97$, $P < 0.001$). Further pairwise comparisons confirmed that pretreatment with LY354740 at 3 and 10 mg/kg (but not at 1 mg/kg) dose-dependently increased the proportion of neurons with decrease responses compared with the vehicle/vehicle group (Fig. 3A, LY 1 mg/kg/Veh vs. Veh/Veh, $\chi^2 = 0.62$, $P > 0.05$; LY 3 mg/kg/Veh vs. Veh/Veh, $\chi^2 = 8.63$, $P < 0.05$; LY 10 mg/kg/Veh vs. Veh/Veh, $\chi^2 = 70.26$, $P < 0.001$). The temporal profile of changes in firing rate for neurons with increase or decrease responses is depicted in Fig. 3B. There was a significant increase in the duration of decrease responses for the LY354740 (10 mg/kg) group [Table 2, $F(3,143) = 34.72$, $P < 0.001$, ANOVA].
Taken together, these data show that LY354740 dose-dependently inhibits the firing of the majority of PFC neurons.

**LY354740 pretreatment blocks the MK801-induced increases in firing rate**

MK801 at 0.1 mg/kg produced a robust increase in the firing rate of the majority of PFC neurons. MK801 increased the percentage of neurons with an increase response (Fig. 4A, compared with Veh/Veh, $\chi^2 = 75.41, P < 0.001$) as well as the average duration of increase responses [Table 2, $F(4,163) = 19.35, P < 0.001$, ANOVA; $P < 0.001$, Bonferroni post hoc test].

Pretreatment with the lower doses of LY354740 (1 and 3 mg/kg) significantly decreased the number of MK801-induced increase responses (Fig. 4A, LY 1 mg/kg/MK801 vs. Veh/MK801, $\chi^2 = 50.59, P < 0.001$; LY 3 mg/kg/MK801 vs. Veh/MK801, $\chi^2 = 52.93, P < 0.001$; LY 10 mg/kg/MK801 vs. Veh/MK801, $\chi^2 = 83.41, P < 0.001$). The highest dose of LY354740 (10 mg/kg) significantly increased the number of decrease responses even in the presence of MK801 (Fig. 2C, LY 10 mg/kg/MK801 vs. Veh/Veh, $\chi^2 = 42.30, P < 0.001$).

The temporal profiles of changes in firing rates were analyzed separately for neurons with increase or decrease responses to MK801 (Fig. 4B). In neurons with increase responses, there was a significant overall effect of group [$F(4,163) = 6.67, P < 0.001$, ANOVA with time as repeated measure]. Further comparison with vehicle/vehicle control group showed a significant effect for MK801 [$F(1,96) = 7.2, P < 0.01$] that was dose-dependently blocked by LY354740 [1 mg/kg, $F(1,82) = 5.56, P < 0.05$; 3 mg/kg, $F(1,102) = 3.75, P < 0.05$; 10 mg/kg; $F(1,87) = 18.2, P < 0.001$]. In neurons with decrease responses, there was also a significant overall group effect [$F(4,135) = 8.75, P < 0.001$], which resulted from the significantly stronger decrease responses in the LY354740 10 mg/kg/MK801 group. All 3 doses of LY354740 attenuated the effect of MK801 on duration of increase responses [Table 2, $F(4,163) = 19.35, P < 0.001$, ANOVA] and decrease responses [$F(4,135) = 9.69, P < 0.001$].

MK801 produced the expected behavioral stereotypy in rats, which involved repeated ambulation, rearing, head wag, and oral movements. Neither vehicle nor LY354740 induced stereotypical behavior (data not shown). However, pretreatment with LY354740 dose-dependently blocked the MK801-induced increase in stereotypy score (Fig. 4C).

**LY354740 posttreatment reverses MK801-induced changes in firing rate and behavior**

Considering the potent inhibitory effect of pretreatment with LY354740 on MK801-induced increase in firing activity, we sought to determine whether mGlu2/3 receptor activation is also capable of blocking the effects of the NMDA antagonist after it has exerted its maximal effects on neuronal activity. Thus in a second set of experiments we posttreated animals with LY354740 50 min after the administration of MK801. All groups treated with MK801 showed an increase in the proportion and average duration of increase responses during the 50-min period before the second injection (data not shown). Because increase responses constitute the main effect of the NMDA antagonist, we focused on comparing the characteristics of this type of response between different groups after the second injection.

Posttreatment with LY354740 reduced the effects of MK801 on PFC unit activity. In the MK801/Veh group a large number of neurons had a sustained increase in firing rate (Fig. 5A, compared with Veh/Veh, $\chi^2 = 18.87, P < 0.001$), LY354740 at 10 mg/kg (but not at 1 mg/kg) reversed this effect of MK801 and significantly increased the number of decrease responses (MK/LY 10 mg/kg vs. MK/Veh, $\chi^2 = 136.24, P < 0.001$; MK/LY 10 mg/kg vs. Veh/Veh, $\chi^2 = 115.83, P < 0.001$).

Analysis of the temporal profile of changes in the firing rate of neurons with increase responses revealed a significant overall effect for group in all 3 types of neurons [Fig. 5B, $F(3,223) = 48.33, P < 0.001$, ANOVA with time as repeated measure]. In fact, the strong excitation induced by MK801 [vs. Veh/Veh control, $F(1,97) = 9.028, P < 0.005$] was significantly attenuated by the lower dose of LY354740 [1 mg/kg vs. MK801/Veh, $F(1,120) = 8.80, P < 0.005$] and was changed to a predominant decrease response by the higher dose of LY354740 [vs. MK801/Veh, $F(1,156) = 145.52, P < 0.001$]. There was an increase in the duration of increase responses to
LY354740 inhibits the MK801-induced changes in spontaneous bursting

Concurrent with its excitatory effect on firing rate, MK801 changed spontaneous bursting activity. Figure 6A shows burst parameters measured during the period after the second injection. Data for all groups have been shown but comparisons were made separately for groups in LY354740 pretreatment and posttreatment experiments. Because separate analysis of the bursting data for neurons with increase, decrease, or no change firing rate responses showed a similar pattern of effects in each group, data for all neurons in each treatment group were combined. MK801 decreased the percentage of spikes in bursts \( F(7,705) = 18.75, P < 0.001 \), one-way ANOVA; post hoc comparison \( P < 0.001 \), the mean number of bursts/min \( F(7,705) = 9.31, P < 0.001 \); post hoc \( P < 0.001 \), and mean number of spikes per burst \( F(7,705) = 9.84, P < 0.001 \); post hoc \( P < 0.05 \). Taken together, these effects suggest that the MK801-induced increase in firing rate is associated with an increase in random spikes (Poisson) that do not occur in bursts.

Further post hoc analysis showed that LY354740 pretreatment dose-dependently reversed the effects of MK801 on burst parameters. In the LY354740 posttreatment experiments, the effects of MK801 on bursting parameters was reversed by both doses of LY354740 [spikes/bursts percentage, \( F(3,433) = 18.69, P < 0.001 \); bursts/min, \( F(3,433) = 12.34, P < 0.01 \); spikes per bursts, \( F(3,433) = 6.75, P < 0.001 \)]. Because the design of this latter experiment allowed assessment of the effect of LY354740 on the individual neurons previously exposed to MK801, we used the data for percentage of spikes in bursts from this experiment to analyze the temporal profile of change in bursting activity. For individual neurons, the normalized percentage of spikes per burst for each of the

**TABLE 2.** Comparison of the average duration (min) of significant increase or decrease changes in firing rate in response to different treatments (after second injection)

<table>
<thead>
<tr>
<th>Group</th>
<th>Increase Responses</th>
<th>Decrease Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1—Vehicle/Vehicle (20-min interval)</td>
<td>44.31 ± 6.8</td>
<td>34.6 ± 5.9</td>
</tr>
<tr>
<td>2—LY 1/Vehicle</td>
<td>38.75 ± 12.73</td>
<td>24.11 ± 6.31</td>
</tr>
<tr>
<td>3—LY 3/Vehicle</td>
<td>51.38 ± 9.1</td>
<td>50.23 ± 11.14</td>
</tr>
<tr>
<td>4—LY 10/Vehicle</td>
<td>30.7 ± 8.2</td>
<td>105.14 ± 4.88¹</td>
</tr>
<tr>
<td>5—Vehicle/MK801</td>
<td>95.3 ± 4.88¹</td>
<td>47.1 ± 11.4</td>
</tr>
<tr>
<td>6—LY 1/MK801</td>
<td>66.2 ± 5.66</td>
<td>55.8 ± 13.9</td>
</tr>
<tr>
<td>7—LY 3/MK801</td>
<td>51.22 ± 6.017b</td>
<td>69.54 ± 8.1²</td>
</tr>
<tr>
<td>8—LY 10/MK801</td>
<td>40.5 ± 6.3b</td>
<td>87.8 ± 6.2²</td>
</tr>
<tr>
<td>9—Vehicle/Vehicle (50-min interval)</td>
<td>28.26 ± 4.94</td>
<td>38.05 ± 5.32</td>
</tr>
<tr>
<td>10—MK801/Vehicle</td>
<td>106.31 ± 6.41⁴</td>
<td>27.37 ± 4.35</td>
</tr>
<tr>
<td>11—MK801/LY 1</td>
<td>71.63 ± 8.78³b</td>
<td>50.9 ± 13.5</td>
</tr>
<tr>
<td>12—LY 10/MK801</td>
<td>35.24 ± 2.43³⁵</td>
<td>71.25 ± 6.84⁴a</td>
</tr>
</tbody>
</table>

¹ \( P < 0.05 \), compared to corresponding vehicle/vehicle control group. ² \( P < 0.05 \), compared to corresponding vehicle + MK801 group.

MK801-induced behavioral stereotypy was dose-dependently reversed by LY354740 posttreatment (Fig. 5C). We previously showed that there is a high correlation between MK801-induced stereotypy and increased firing in PFC (Jackson et al. 2004). To assess the degree of correlation between MK801 reversal of the behavioral and electrophysiological effects of MK801, we calculated the correlation between stereotypy score and average firing rate of the neurons that showed an early increase response to MK801 injection in each individual animal. As shown in Fig. 5D, there was poor correlation between stereotypy score and firing rate in vehicle/vehicle group, but high correlations in all of the other 3 groups (MK801 plus vehicle, LY 1 or 10 mg/kg). Of note, in the vehicle/vehicle control group, the absence of stereotypy led to a flat regression line, whereas in other groups treated with MK801 the presence or absence of stereotypy was closely correlated with PFC firing activity (see example shown in Fig. 5E).

**FIG. 3.** Effects of LY354740 and vehicle injection on spontaneous firing rate of PFC neurons in awake rats. Animals received 2 injections, the first being either vehicle or LY354740, and the second injection being vehicle 20 min later. A: proportion of neurons with sustained changes (≥10 min) in firing rates after second drug injection compared with baseline. Percentage of neurons with increase, decrease, or no change in firing rate is shown. There was a significant increase in the number of neurons with decrease responses for higher doses of LY354740 (see Results). B: temporal profile of the firing activity of neurons that showed a significant increase (top panel) or decrease (bottom panel) in firing rate after LY354740 or vehicle injection. LY354740 was given at one of 3 doses (1, 3, or 10.0 mg/kg), indicated in the legend as LY 1, LY 3, and LY 10, respectively. First injection is given at time 0. Arrows indicate the time of each injection.

MK801 [Table 2, \( F(3,223) = 39.20, P < 0.001 \), ANOVA, \( P < 0.001 \), Bonferroni post hoc test]. This effect was attenuated dose-dependently by LY354740.
MK801 decreases the variability of PFC unit firing

Cortical neurons of awake animals show a high level of instantaneous firing rate variations that may be a result of synaptic background activity (Holt et al. 1996). Here 2 measures, CV and CV2, were used to assess the drug-induced changes in variability of spiking activity. The data for the LY354740 posttreatment experiments are shown because they allow comparison of drug-induced changes in variability within individual spike trains (Fig. 7A). However, the results for the LY354740 pretreatment experiments showed the same pattern of effects (data not shown). In all groups, the average baseline values for CV were higher than 1.0, the value expected for a Poisson process. These values of CV are similar to what has previously been reported in other cortical areas (Holt et al. 1996; Lee et al. 1998; Softky and Koch 1993; Vogels et al. 1989). This high level of irregularity of spiking in PFC did not significantly change in the control group throughout the experiment (analyzed as separate 30-min epochs). However, MK801 significantly decreased the mean CV [MK/LY 1 mg/kg, 4.106, P < 0.001, ANOVA]. We then calculated CV2 as a local measure of variability that is independent of gradual changes in firing rate. Mean CV2 of the vehicle/vehicle group was very stable throughout the length of the recordings (Fig. 7B). However, there was a significant decrease in CV2 after MK801 administration compared with baseline period that was also significantly different from that in the vehicle/vehicle group [F(5,528) = 16.75, P < 0.001]. This finding shows the MK801-induced decrease in spiking variability cannot be accounted for by changes in the firing rate. Posttreatment with LY354740 at both 1 and 10 mg/kg reversed the effect of MK801 on mean CV [MK/LY 1 mg/kg, F(5,366) = 4.518, P < 0.001; MK/LY 10 mg/kg, F(5,792) = 22.185, P < 0.001] and mean CV2 [MK/LY 1 mg/kg, F(5,366) = 15.127, P < 0.001; MK/LY 10 mg/kg, F(5,792) = 96.04, P < 0.001]. The higher dose of LY354740 significantly increased the mean CV and CV2 above the baseline level, consistent with its effect when given as a pretreatment (data not shown).

**Discussion**

The present findings demonstrate that activation of mGlu2/3 receptors reverses the disruptive effects of NMDA receptor hypofunction on spontaneous spike activity and bursting of PFC neurons in awake rats. These electrophysiological changes coincided with blockade of the MK801-induced behavioral stereotypy by the mGlu2/3 receptor agonist. The latter finding is consistent with previous behavioral studies (Cartmell et al. 1999; Moghaddam and Adams 1998) and further suggests that mGlu2/3 receptor activation is effective in reversing the behavioral and cellular effects of NMDA receptor blockade even after these behaviors have been fully expressed. Treatment with LY354740 in the absence of MK801 led to a dose-dependent decrease in spontaneous firing of PFC neurons.
Effects of vehicle and MK801

Injection of MK801 (0.1 mg/kg), either 20 min after vehicle or as the first injection, significantly increased the firing rate of the majority of PFC neurons and, at the same time, modified the bursting pattern of PFC neurons by decreasing the relative number of bursts and the number of spikes in bursts. This finding replicates our recent report showing that MK801 produces a dose-dependent pattern of change in PFC neuronal firing of awake animals (Jackson et al. 2004). Most neurons had a brief but significant response to the vehicle injection, which may be caused by the stress associated with receiving an ip injection.

The increase in random firing in response to MK801 may be attributable to the preferential blockade of NMDA receptors on inhibitory GABAergic interneurons, leading to a disinhibition of glutamate neurons and a subsequent increase in PFC glutamate release (Feenstra et al. 2002; Lorrain et al. 2003; Moghaddam and Adams 1998; Moghaddam et al. 1997; Olney and Farber 1995). This increase in glutamate release in turn can activate cortical AMPA (α-amino-3-hydroxy-5-methyl-4-isox-
azolepropionic acid) receptors and potentiate the spontaneous activity of cortical neurons. The potentiation in firing rate we observed, however, was accompanied by a profound reduction in burst activity. The bursting activity, as measured here, represents periods in a spike train that have a higher discharge rate than do other periods in the same spike train. This pattern of bursting is thought to be associated with higher probability of neurotransmitter release (Cooper 2002). The effect of the NMDA antagonist on spontaneous bursting is in accord with the well-known role of NMDA receptors in sustaining membrane excitability and, specifically, in mediating long-term plastic changes (Dingledine et al. 1986; Malenka and Nicoll 1993; Stoop et al. 2003). Recently, the NMDA antagonist phencyclidine was shown to suppress the dendritic glutamate-induced bursting in PFC slices (Shi and Zhang 2003). Because bursting in cortical areas may be critical for cognitive processes (Cooper 2002; Miller and Cohen 2001; Schultz and Dickinson 2000), the disruption of bursting in PFC by NMDA antagonists may account for the cognitive deficits associated with these compounds.

**Effect of mGlu2/3 receptor agonist LY354740**

Pretreatment with LY354740 at 1 or 3 mg/kg did not produce a significant effect on PFC neuronal activity. However, at the higher dose of 10 mg/kg, LY354740 increased the proportion of decrease responses. The dose-dependent effect of LY354740 on PFC unit firing suggests that the lower doses...
of the agonist may have minimal side effects. However, at higher doses a general depression in PFC activity may be associated with cognitive impairment. This agrees with previous reports that LY354740 produces a dose- and delay-dependent impairment of working memory (Aultman and Moghaddam 2001; Higgins et al. 2004), an effect that is distinct from the memory-enhancing influence of this drug in the presence of an NMDA receptor antagonist (Moghaddam and Adams 1998). The depression of PFC neuronal firing may also play a role in the anxiolytic properties of mGlu2/3 agonists (Ferris et al. 2001; Helton et al. 1998; Spooren et al. 2002). Excessive glutamate release in PFC has been implicated in stressful conditions (Moghaddam 2002) and reduction of this release by mGlu2/3 agonists may provide a mechanism for their anxiolytic effects (Schoepp et al. 2003). Notably, a recent report has shown that LY354740 decreases stress-induced noradrenaline release in PFC, probably through autoregulatory effects on PFC excitatory efferents (Swanson et al. 2004).

LY354740 also produced a dose-dependent effect on bursting properties of PFC neurons such that at the higher dose it increased the percentage of spikes in bursts without changing the total number of firing bursts. Although presynaptic blockade of glutamate release is the most likely cause of LY354740-induced decrease in firing rate (Cartmell and Schoepp 2000; Kilbride et al. 1998; Schoepp et al. 1999), its effect on bursting may involve a postsynaptic modulation of membrane excitability. A recent report on acutely dissociated PFC pyramidal cells suggests that mGlu2/3 agonists produce postsynaptic enhancement of NMDA receptor currents (Tyszkiewicz et al. 2004). These postsynaptic mechanisms may contribute to the increase in relative bursting activity by LY354740.

**Reversal of MK801 effects by LY354740**

Agonists of mGlu2/3 receptors reverse the behavioral and neurochemical effects of NMDA receptor antagonists including hyperlocomotion, behavioral stereotypy, increased PFC glutamate release, and impaired working memory (Cartmell et al. 1999; Lorrain et al. 2003; Moghaddam and Adams 1998), suggesting that these ligands may have therapeutic potential for treating some symptoms of schizophrenia (Krystal et al. 2003; Marek et al. 2000; Marino and Conn 2002; Spooren et al. 2003). Interestingly, we found that both pretreatment and posttreatment with LY354740 blocked the electrophysiological and behavioral effects of MK801. Pretreatment with the lower doses of LY354740 (1 and 3 mg/kg), which had no effect on basal firing rate, significantly reduced the number of neurons with MK801-induced increase responses. Similarly, posttreatment with LY354740, injected after MK801 began to exert its maximal effects on PFC unit firing, reversed the changes in firing activity and dose-dependently decreased the duration of increase responses. LY354740 also influenced the pronounced effects of MK801 on the bursting activity of PFC neurons. Specifically, either pre- or posttreatment with LY354740 inhibited the MK801-induced decrease in the total number of bursts and spikes per burst ratio.

Activation of the mGlu2/3 receptors decreases evoked glutamate release in both in vivo and in vitro preparations (Battaglia et al. 1997; East et al. 1995; Kilbride et al. 1998; Marek and Aghajanian 1998). NMDA receptor antagonists increase glutamate release in the PFC of awake animals (Adams and Moghaddam 1998; Lorrain et al. 2003). This form of evoked glutamate release is also attenuated after pretreatment with the mGlu2/3 agonist LY354740 (Moghaddam and Adams 1998). This putative presynaptic inhibitory effect of LY354740 on glutamate release may play a critical role in reversing the MK801-induced increase in PFC neuron firing rate. On the other hand, the postsynaptic effects of the mGlu2/3 agonist, such as enhancement of NMDA-mediated currents (Tyszkiewicz et al. 2004), most likely account for the normalizing effects of LY354740 on burst activity. The fact that LY354740 restored the normal pattern of firing after MK801 had exerted its physiological (and behavioral) effect further suggests that mGlu2/3 agonists may modulate the activity of cortical networks, a possibility that warrants further investigation with these drugs during the performance of PFC-dependent cognitive tasks.

We observed a significant correlation between the temporal dynamics of stereotypy and excessive PFC neuronal firing, and their reversal by mGlu2/3 receptor activation. Measures of...
NMDA receptor antagonist–induced stereotype are of interest in the context of animal models of schizophrenia because behavioral perseveration and stereotypical movements are prevalent in patients with schizophrenia (Crider 1997; Tracy et al. 1996). Although motor stereotypy is traditionally associated with subcortical, mostly basal ganglia, regions, several recent studies have shown that lesions or functional inactivation of PFC reduces the motor effects of NMDA antagonist treatment (Jentsch et al. 1998; Takahata and Moghaddam 2003). The present findings further confirm that aberrant activity of PFC neurons may be a prime source of behavioral abnormalities in this model.

Treatment with MK801 also decreased the variability of PFC neuronal spiking in a manner that was independent of gradual changes in firing rate (mean CV2). The high level of variability observed in the PFC is similar to what has been previously reported for other cortical areas (Holt et al. 1996; Lee et al. 1998; Softky and Koch 1993; Vogels et al. 1989). Some authors have suggested that the variability in firing rate of cortical neurons increases during functionally important epochs (Baddeley et al. 1997; Lee et al. 1998; Softky and Koch 1993). For example, in monkeys, both CV and CV2 significantly increase during the mnemonic activity of PFC neurons (Compte et al. 2003). Thus the significant decrease in measures of variability after MK801 may reflect a relative loss of flexibility in neuronal responding that may contribute to its detrimental cognitive and behavioral effects. There is a possibility that changes in firing and bursting activity may influence variability measures that reflect the distribution of ISIs in a spike train. However, in spike trains with nonperiodic bursts and irregular interspike intervals, such as those of PFC units, these measures (in particular CV2) may be independent of direct changes in bursting or firing rate. The reversal by LY354740 of decreased spiking variability may play a role in its restoration of cognitive capacities in NMDA antagonist–treated subjects. Further studies are needed to elucidate this mechanism.

In data recorded from awake rats, one concern that needs to be addressed is the possibility that changes in an animal’s motor activity may influence neuronal firing. This is especially a concern in the present study because LY354740 reduces NMDA antagonist–induced hyperlocomotion (Cartmell et al. 1999; Moghaddam and Adams 1998). However, previous studies indicate that PFC neurons generally do not show direct movement-related or spatially tuned patterns of activity (Gemello et al. 2002; Jung et al. 1998; Pouzet 1997). Furthermore, our preliminary data indicate that increased PFC neuronal activity may be detected while the animal is not motorically active [e.g., when animal is undergoing a restraint stress procedure (Jackson and Moghaddam 2004)], whereas decreases or no change in PFC neuronal activity is observed when animals are treated with stimulants such as amphetamine that produce profound motor activation (Homayoun and Moghaddam 2004).

In conclusion, the present results demonstrate that augmenting mGlu2/3 receptor function reduces the disruptive effects of NMDA receptor blockade on unit firing and burst activity of PFC neurons. This influence was accompanied by reversal of NMDA antagonist–induced behavioral stereotypy and was observed both when the mGlu2/3 agonist was administered as a pretreatment and when it was administered after the behavioral effects of MK801 were fully expressed. These findings describe a physiological mechanism for normalization of NMDA receptor antagonist–induced behaviors by mGlu2/3 agonists.

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