Introduction

It is well established that glutamate neurotransmission, through activation of NMDA and AMPA receptors, plays a critical role in cognitive functions including learning, behavioral flexibility, and working and long-term memory (Holscher et al., 1999; Riedel et al., 2003). However, the ubiquitous nature of these receptors limits their potential as direct therapeutic targets for improving cognition. There is accumulating evidence that manipulation of glutamate neurotransmission through G-protein-coupled metabotropic glutamate (mGlu) receptors (Conn and Pin, 1997; Schoepf and Conn, 2002) may influence cortical function, providing a potentially more feasible therapeutic strategy for treating major cognitive pathologies (Moghaddam, 2004). In this regard, group I mGlu receptors, including mGlu1 and mGlu5 receptors, have been of special interest because they are abundant in corticolimbic circuitry (Shigemoto et al., 1993; Romano et al., 1995) and have been implicated in learning and memory (Lu et al., 1997; Balschun et al., 1999; Manahan-Vaughan and Schuetz, 2002). For instance, the mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) impairs working memory and spatial or instrumental learning (Balschun and Wetzel, 2002; Homayoun et al., 2004b; Naie and Manahan-Vaughan, 2004). Mutant mice lacking mGlu5 receptors exhibit disrupted prepulse inhibition, impaired spatial learning and reduced NMDA receptor-dependent long-term potentiation (Lu et al., 1997; Jia et al., 1998; Kinney et al., 2003; Brody et al., 2004). However, the cellular basis for the behavioral effects of a system-wide reduction in mGlu5 receptor function is not well understood.

A primary mechanism by which mGlu5 receptors influence cortical function may be through interactions with NMDA receptors (Doherty et al., 1997; Alagarsamy et al., 1999). For example, activation of the mGlu5 receptor facilitates, while its inhibition attenuates, NMDA-evoked responses (Pisani et al., 1997; Ugolini et al., 1997; Attucci et al., 2001; Mannioni et al., 2001; Movsesyan et al., 2001). At a behavioral level, antagonists of mGlu5 and NMDA receptors have additive detrimental effects on learning, working memory and sensorimotor gating (Henry et al., 2002; Kinney et al., 2003; Campbell et al., 2004; Homayoun et al., 2004b). These findings have led to speculations that pharmacological manipulation of mGlu5 receptors may provide a novel strategy to influence NMDA-dependent cognitive functions.

Previous physiological characterizations of mGlu5 receptors have been performed in in vitro preparations, which limit the extrapolation of those data to cognitive functioning. A better understanding of the role of mGlu5 receptors in behaviorally (and clinically) relevant contexts may be achieved by studying their contribution to the spontaneous activity of ‘awake’ cortex. Thus, we studied the effects of systemic mGlu5 receptor inhibition and the synergistic interaction of mGlu5-NMDA receptors on spontaneous activity of cortical neurons in awake animals. Ensemble unit recording was used to record extracellular activity from the medial prefrontal cortex (mPFC) because this region in the rat is critical for cognitive functions. Therefore, we studied the effects of systemic mGlu5 receptor inhibition and the synergistic interaction of mGlu5-NMDA receptors on spontaneous activity of cortical neurons in awake animals. Ensemble unit recording was used to record extracellular activity from the medial prefrontal cortex (mPFC) because this region in the rat is critical for cognitive functions.

Materials and Methods

Subjects

A total of 14 adult male Sprague-Dawley rats, weighing 340–420 g, were used in this study. Animals were individually housed on a 12 h light/dark cycle (lights on at 07:00 h), and experiments were performed during the light phase. 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.

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Metabotropic glutamate 5 (mGlu5) receptors have been recently implicated in prefrontal cortex (PFC)-dependent executive functions because inhibition of mGlu5 receptors impairs working memory and worsens cognitive-impairing effects of NMDA receptor antagonists. To better understand the mechanisms by which mGlu5 receptors influence PFC function, we examined the effects of selective mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP), given alone or in combination with the NMDA receptor antagonist MK801, on ensemble single unit activity in the medial PFC (mPFC) of behaving rats. MK801 decreased the spontaneous burst activity of the majority of mPFC neurons. This inhibition was selective for the most active cells because greater decreases were observed in neurons with higher baseline firing rates. MPEP augmented the effects of MK801 on burst activity, variability of spike firing and random spike activity. These findings demonstrate that in awake animals mGlu5 receptors regulate the function of PFC neurons by two related mechanisms: (i) rate-dependent excitatory influence on spontaneous burst activity; and (ii) potentiation of NMDA receptor mediated effects on firing rate and burst activity. These mechanisms support the idea that modulation of mGlu5 receptors may provide a pharmacological strategy for fine-tuning the temporal pattern of firing of PFC neurons.

Keywords: ensemble single unit recording, metabotropic glutamate receptors, NMDA receptor hypofunction, prefrontal cortex, schizophrenia

Bursting of Prefrontal Cortex Neurons in Awake Rats is Regulated by Metabotropic Glutamate 5 (mGlu5) Receptors: Rate-dependent Influence and Interaction with NMDA Receptors

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Electrophysiological Recording

Chronically implanted microelectrode arrays (NB Labs, Denison, TX) were used for all single unit recordings (Homayoun et al., 2004a). Arrays consisted of eight 50-μm-diameter Teflon-insulated, stainless steel wires arranged in a 2 × 4 pattern measuring −0.25 × 0.7 mm. Electrode arrays were chronically implanted under halothane anesthesia in mPFC (target coordinates for the center of the array at AP 3.0, ML 0.7 and DV −3.5; Fig. 1), according to the atlas of Paxinos and Watson (1986). Animals were allowed 1 week to recover from surgery before experiments were started. All recordings were performed in the animal’s home cage (clear polycarbonate 44 × 22 × 42 cm) with a modified open top extending an additional 42 cm above the home cage. Animals were connected to a FET headstage (NB Labs) by means of lightweight cabling that passed through a 24-channel commutator (NB Labs) and allowed the animal unrestricted movement during recording. Extracellular unit activity was recorded using multiple channel amplifiers with 500× gain and 220–59 kHz band pass filters (Plexon Inc., 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 movement artifacts, 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 that included valley seeking and k-means clustering methods, computer-generated waveform template matching and manual checking of the quality of single unit isolation (for a review, see Lewicki, 1998). 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 due to noise or similar waveforms. An absolute refractory period of at least 1.1 ms was used to select single units. Typically, two or three neurons were isolated from each electrode. Based on previously reported criteria about firing rate (FR) and autocorrelogram statistics (McCormick et al., 1985; Jung et al., 1998), neurons with fast firing rate (FR > 10 Hz) may be interneurons. Therefore, we divided the neurons into regular firing (FR < 10 Hz, sporadic firing pattern in autocorrelogram, putative pyramidal neurons) and fast firing (FR > 10, regular firing pattern, putative interneurons) units. However, because only eight neurons met the criteria of fast firing neurons, reliable statistical analysis could not be performed for this subgroup. Thus, all reported results are based on regular firing single units.

Experimental Procedures

After 1 week of recovery, animals were transferred daily to the recording chamber and were connected to the headstage for 2 h to habituate to the recording environment. The recording sessions started after 1 week of acclimation. Each animal received one treatment (one or two injections) during a recording session. Animals were exposed to a maximum of three treatment sessions, each separated by 1 week. The order of treatments was pseudo-randomized such that each drug was administered to at least four animals and each animal received each treatment only once. Each session consisted of a 1 h habituation period, 30 min baseline recording before the first injection and up to 2 h recording after the last injection. The NMDA receptor antagonist MK801 (Sigma-RBI, St Louis, MO) and the selective specific mGlu5 receptor antagonist MPEP (Tocris Cookson, Bristol, UK) were both dissolved in saline vehicle (0.9%) and injected intraperitoneally. The effect of 10 mg/kg MPEP was compared with a single vehicle injection, both administered after 30 min of baseline recording. The effects of combined administration of MPEP and MK801 were evaluated in two separate sets of experiments. In the first set, MPEP (10 mg/kg; high dose) or vehicle were administered at min 30 followed by 0.1 mg/kg MK801 at min 50. A double vehicle injection group was used as control. In the second set, MPEP (3 mg/kg; low dose) or vehicle were administered at min 30, followed by MK801 (0.01 mg/kg or vehicle) at min 50. A summary of treatment schedule is depicted in Table 1. The doses and time intervals for MPEP and MK801 administrations were chosen based on our previous findings (Homayoun et al., 2004b; Jackson et al., 2004).

Data Analysis

Electrophysiological data was 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. Changes in firing rate were measured by comparing each normalized rate histogram bin after drug administration to the normalized baseline firing rate. A significant change in firing rate was
defined as two consecutive 5 min bins exceeding the 99% confidence intervals of the baseline. Termination of a significant response also required two consecutive 5 min bins within the 99% confidence intervals of the baseline. This criterion was chosen because we were interested in sustained effects of different treatments on the spontaneous firing of mPFC neurons (Jackson et al., 2004). Neuronal responses were divided into one of three response types depending on whether they showed a significant increase (type 1; increase), a significant decrease (type 2; decrease) or no change (type 3; no change) in spontaneous firing rate. To avoid the transient effects of the injection, windows of analysis started 10 min after the injection of interest (the single injection in the first two groups, the second injection in other groups; see Table 1). Neurons were grouped by response types for further analysis.

The Pearson $\chi^2$ 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 a two-way repeated measures analysis of variance (ANOVA) with time as the repeated measure. Wherever a significant effect was observed, further analysis between two groups was carried out using a one-way ANOVA and the Bonferroni post-hoc test. A $P$ value less than 0.05 was used as criterion of significance in this and all subsequent analysis. In addition, the average magnitude of increase and decrease responses were calculated and compared across groups using a one-way ANOVA with the Bonferroni post-hoc test. To assess the effect of baseline firing activity on post-drug firing rate, we plotted the average post-drug firing rate of each neuron versus its baseline firing rate. We then used the median value of baseline firing rate for all neurons (1.66 Hz) to divide the neuronal population into two subsets and compared the average changes in firing rate for all neurons in each subset using the Student’s $t$ test. A similar analysis was used to compare the effects of higher versus lower baseline firing rate on changes in bursting activity (see below).

Bursts may be defined as periods in a spike train that have a higher discharge rate than do surrounding periods in the same spike train (Kaneoke and Vitek, 1996). In the present study, bursts and burst-related statistics were determined using the Poisson surprise method of Legendy and Salzman (1985) as implemented in NeuroExplorer. This method of burst detection is well suited to our data where we are recording spontaneous discharges of neurons without external triggering, and we desire to isolate single events from a continuum of events in a spike train (Homayoun et al., 2004a, Jackson et al., 2004). It should be emphasized that bursts detected by this method are periods of ‘non-Poisson high frequency firing’ and may represent a combination of increased afferent-driven and intrinsic spiking activity. However, PFC neurons recorded from awake animals do not show periodic intrinsic bursts similar to those observed in thalamic or dopaminergic neurons. Thus, the functional definition of bursts used here is a measure of relative clustering of spikes that most likely reflects the afferent synaptic effects on cortical units. Regardless of mechanisms of induction, these periods of compressed firing activity are believed to be of functional significance in relaying information in the neuronal networks (Cattaneo et al., 1981; Schultz et al., 2000; Harris et al., 2001). 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 with the same mean firing rate. Thus, this analysis is not sensitive to changes in mean firing rate. Burst parameters measured for each spike train included the number of bursts per min, the percentage of spikes that occur in bursts and the mean number of spikes per burst. Comparisons of burst parameters between groups were made using a one-way ANOVA with the Bonferroni post-hoc test. To further analyze the temporal profile of changes in bursting activity, histograms of relative changes in bursting rate (300 s bins, normalized to baseline rate for each individual neuron) were constructed and averaged for all neurons in each treatment group. A two-way ANOVA with time as repeated measure was used to detect significant differences and was followed by post-hoc analysis where justified.

The temporal pattern of cortical neuronal discharges shows a high level of variability in awake animals (Softky and Koch, 1993; Holt et al., 1996; Lec et al., 1998). This is thought to result primarily from instantaneous changes in firing frequency that are superimposed on an otherwise steady mean firing rate. To assess the variability of firing for each spike train, we calculated the coefficient of variation (CV) of ISIs by dividing the standard deviation (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, in the spike trains that demonstrate changes in mean firing rate, CV is not a reliable measure of variability. Thus, we calculated a second local measure of variability, CV2, that provides a more reliable measure of intrinsic variability of spiking processes independent of gradual changes in firing rate (Holt et al., 1996; Compte et al., 2003). In brief, CV2 was computed by assessing the SD and mean firing for each two adjacent ISIs in the spike train and calculating the two-point coefficient of variation $[CV2 = 2(ISI2 - ISI1)/(ISI2 + ISI1)]$. The CV2 for each pair of adjacent ISIs was then plotted against the mean of those two 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 window of analysis. The effect of each drug treatment on spike variability was assessed by within-group comparisons of the mean CV and CV2 values for all neurons in that group for separate 30 min epochs using a one-way ANOVA with the Bonferroni post-hoc test. Comparison between different groups was made using a two-way ANOVA with treatment and epoch as factors.

### Behavioral Stereotypy

Stereotypical behavior was rated during the electrophysiological recording every 5 min, as described previously (Adams and Moghaddam, 1998). In brief, animals received a score of 1 for the repeated presence of each of the following behaviors: ambulation, turning, head wagging, grooming, snuffling up or down, digging, rearing, and mouth movement or jaw tremor. Scores for different repetitive behaviors at each 5 min point were summed and temporal profiles of stereotypy behavior across groups were compared using a one-way ANOVA with time as the repeated measure, followed by the Bonferroni post-hoc test. To compare two groups, a two-way ANOVA with time and treatment as factors was used. To assess the correlation between behavioral and electrophysiological effects of the drugs, we compared the stereotypy score during each 5 min bin with the average firing rate during the same 5 min bin for each rat by using Pearson’s correlation with the Bartlett $\chi^2$ test for significance. For the latter analysis we used only the neurons with an increase firing response (Jackson et al., 2004).

### Histology

Animals were anesthetized with chloral hydrate and intracardially perfused with saline followed by 10% buffered formalin. Fixed brains were stained with cresyl violet and viewed under a light microscope to confirm electrode placement. Electrodes in each recording array

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Injection 1 (min)</th>
<th>Injection 2 (min)</th>
<th>Window of analysis (min)</th>
<th>( n )</th>
<th>Baseline firing rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle (30)</td>
<td>-</td>
<td>40-150</td>
<td>92</td>
<td>1.90 ± 0.31</td>
</tr>
<tr>
<td>2</td>
<td>MPEP 10 mg/kg (30)</td>
<td>-</td>
<td>40-150</td>
<td>69</td>
<td>2.06 ± 0.27</td>
</tr>
<tr>
<td>3</td>
<td>Vehicle (30)</td>
<td>Vehicle (50)</td>
<td>60-150</td>
<td>107</td>
<td>2.38 ± 0.28</td>
</tr>
<tr>
<td>4</td>
<td>Vehicle (30)</td>
<td>MK801 0.1 mg/kg (50)</td>
<td>60-150</td>
<td>118</td>
<td>2.66 ± 0.34</td>
</tr>
<tr>
<td>5</td>
<td>MPEP 10 mg/kg (30)</td>
<td>MK801 0.1 mg/kg (50)</td>
<td>60-150</td>
<td>67</td>
<td>1.96 ± 0.19</td>
</tr>
<tr>
<td>6</td>
<td>Vehicle (30)</td>
<td>MK801 0.01 mg/kg (50)</td>
<td>60-150</td>
<td>85</td>
<td>2.46 ± 0.30</td>
</tr>
<tr>
<td>7</td>
<td>MPEP 3 mg/kg (30)</td>
<td>Vehicle (50)</td>
<td>60-150</td>
<td>84</td>
<td>1.69 ± 0.21</td>
</tr>
<tr>
<td>8</td>
<td>MPEP 3 mg/kg (30)</td>
<td>MK801 0.01 mg/kg (50)</td>
<td>60-150</td>
<td>105</td>
<td>1.66 ± 0.25</td>
</tr>
</tbody>
</table>

The timing of each injection is indicated as minutes after the start of the recording session. The window of analysis indicates the period after the start of recording that was used to compare the effects of various treatments on electrophysiological measures. \( n \) indicates the number of neurons in each group. There was no overall significant group effect for the baseline firing rates.
covered a 0.25 × 0.7 mm area. As shown in Figure 1, recordings were made in ventral prelimbic and dorsal infralimbic cortex.

Results

A total of 727 regular firing single units from 14 animals were isolated during 34 recording sessions. Comparison of baseline firing rates did not show any significant differences between groups [Table 1, one-way ANOVA, \( F(7,717) = 1.97, P > 0.05 \)]. Based on the sustained changes in firing rate (for at least 10 min, see methods) in the post-injection period, neuronal responses were classified as increase, decrease or no change.

The Effects of MPEP Treatment

Representative examples of the effects of vehicle or 10 mg/kg MPEP on the firing rate of mPFC neurons are depicted in rate histograms (Fig. 2A,B). 10 mg/kg MPEP significantly increased the number of neurons that displayed a decrease in firing rate (Fig. 2C, \( \chi^2 = 37.27, P < 0.001 \)). Comparison of the temporal profile of significant responses after vehicle and MPEP showed a significant difference between decrease responses [Fig. 2D, lower panel, two-way ANOVA with time as repeated measure, treatment, \( F(1,50 = 14.71), P < 0.001 \); time, \( F(29,1450) = 11.64, P < 0.001 \); treatment × time interaction, \( F(29,1450) = 3.54, P < 0.001 \)]. There was no significant difference between increase responses [Fig. 2D, upper panel]. In general, decrease responses induced by MPEP were larger in magnitude (Table 2, \( t \)-test, \( P < 0.05 \)) and of longer duration (Table 2, \( P < 0.05 \)) compared with the vehicle control group.

Using the Poisson surprise method to detect spontaneous bursts of activity, we found that 10 mg/kg MPEP decreased the number of bursts during the post-drug period compared with the vehicle group (mean ± SEM: vehicle, 2.77 ± 0.13, 10 mg/kg MPEP, 1.37 ± 0.16, \( t \)-test, \( P < 0.05 \)). There was no significant difference in bursting rate during the baseline period (vehicle, 2.88 ± 0.32, 10 mg/kg MPEP, 2.59 ± 0.25, \( P > 0.05 \)). MPEP also significantly decreased the percentage of spikes in bursts (\( P < 0.05 \)) and the average number of spikes per burst (\( P < 0.05 \), Fig. 2E). Comparing the baseline values for these measures did not show any significant difference between groups (\( P > 0.05 \), data not shown). Figure 2F depicts the temporal profile of changes in bursting rate for all neurons. Since different neurons had various levels of baseline bursting activity, we normalized the responses relative to baseline for each neuron. A two-way ANOVA with time as repeated measures revealed a significant decrease in average bursting activity in MPEP-treated neurons compared with vehicle-treated neurons [treatment, \( F(1,159 = 30.54), P < 0.001 \); time, \( F(29,4611) = 8.32, P < 0.001 \); treatment × time interaction, \( F(29,4611) = 6.46, P < 0.001 \)]. This effect started immediately after MPEP injection and lasted for about 80 min. We further examined whether the significant decrease in firing rate after MPEP was caused by a decrease in the number of bursts. For this purpose, we recalculated the firing rates after excluding the bursts. This calculation led to firing rates that were not significantly different between vehicle and 10 mg/kg MPEP (Fig. 2G, \( P > 0.05 \)), suggesting that decreased firing following MPEP is due to a loss of bursting activity and not single spike firing.

The effect of MPEP depended on the initial baseline firing rate of neurons. As demonstrated in the scatter plot of post-drug versus baseline firing rates (Fig. 3A), the majority of neurons with a higher baseline firing rate decreased their firing rate in response to 10 mg/kg MPEP. In comparison, in the vehicle group the distribution of significant responses was not dependent on the baseline firing rate (Fig. 3B). To quantify this effect, we divided the neurons into two groups based on their baseline firing rates being higher or lower than 1.66 Hz, the median baseline value for the whole population of recorded neurons, and compared the mean relative change in firing rates after drug treatment (Fig. 3C). Approximately 41% of neurons in the vehicle group and 43% in MPEP group had a baseline firing rate higher than the overall median. There was no significant difference based on the baseline firing rate in the response of vehicle-treated neurons (\( t \)-test, \( P > 0.05 \)). In contrast, the effect of MPEP was significant only in neurons with a higher than median basal activity (\( P < 0.05 \)).

Changes in bursting activity of individual neurons in response to MPEP were also related to their baseline firing rates. As shown in Figure 3D, while MPEP decreased the percentage of spikes in bursts in the majority of neurons, a larger decrease was observed in neurons with a higher baseline firing rate. In comparison, in the vehicle group changes in bursting activity of individual neurons were not dependent on baseline firing activity (Fig. 3E). Thus, in the MPEP group, neurons which had a baseline firing rate higher than the overall median had a significantly larger decrease in bursting compared to neurons with lower baseline activity (Fig. 3F, \( P < 0.05 \)).

The Effects of Combined MPEP and MK801 Treatment

Based on previously observed synergistic interactions between NMDA and mGlu5 receptor antagonists on behavior (Campbell et al., 2004; Homayoun et al., 2004b), we hypothesized that pre-treatment with MPEP would potentiate the effects of MK801 on mPFC neuronal firing. Subanesthetic doses of MK801 (0.1–0.3, but not 0.01 mg/kg) significantly increase mPFC firing rate with a concomitant decrease in bursting activity (Jackson et al., 2004). Pre-treatment with 10 mg/kg MPEP exaggerated the increase responses induced by 0.1 mg/kg MK801. Representative rate histograms are shown in Figure 4A,B. Although this dose of MPEP did not produce an additive increase in the percentage of neurons with an increase response to MK801 (Fig. 4C, \( \chi^2 = 5.11, P > 0.05 \)), the analysis of temporal profile of firing rates indicated that 10 mg/kg MPEP + 0.1 mg/kg MK801 produced a larger response than the vehicle + MK801 group [Fig. 4D, two-way ANOVA with time as repeated measure, treatment, \( F(2,153) = 11.27, P < 0.001 \); time, \( F(29,4437) = 26.50, P < 0.001 \); treatment × time interaction, \( F(58,4437) = 6.58, P < 0.001 \)]. Specifically, MPEP pretreatment significantly increased the average magnitude [Table 2, one-way ANOVA, \( F(2,153) = 13.31, P < 0.001 \), post-hoc \( P < 0.001 \)] and duration [\( F(2,153) = 18.31, P < 0.001 \), post-hoc \( P < 0.01 \)] of increase responses to MK801, compared with vehicle-pretreatment. There was no significant effect in decrease responses.

Next we examined the interaction between lower doses of MK801 (0.01 mg/kg) and MPEP (3 mg/kg) because the combination of these doses leads to additive cognition-impairing effects (Homayoun et al., 2004b). Both MPEP at 3 mg/kg and MK801 at 0.01 mg/kg produced increase responses in ~40% of neurons (Fig. 4E). There was no significant difference between the temporal profile of increase responses induced by either drug alone, compared with that of the vehicle + vehicle
Figure 2. The effect of 10 mg/kg MPEP on mPFC firing rate. (A, B) Representative rate histograms of mPFC unit activity after a single injection (arrows) of vehicle (A) or 10 mg/kg MPEP (B). Five-minute histogram bins are shown along the x-axis. Each panel shows an individual single unit. (C) Effect of vehicle or MPEP injections on the proportion of neurons that showed a sustained change in firing rate compared with baseline (see Materials and Methods). The majority of neurons displayed no change in response to vehicle. MPEP decreased the proportion of no change responses and increased the number of decrease responses. (D) The temporal profile of the firing activity of neurons that had a significant increase (upper panel) or decrease (lower panel) in firing rate after MPEP or vehicle injection. Injection time is shown as min 0 on the x-axis (arrow). There was a significant difference between decrease responses after MPEP compared with vehicle. (E) MPEP decreased the spontaneous bursting activity of mPFC neurons as detected by Poisson surprise method (see Materials and Methods). MPEP significantly reduced the percentage of spikes in bursts and the average number of spikes per burst. (F) Time course of MPEP’s effect on the rate of bursting. Results are depicted as changes in bursting rate in a normalized burst rate histogram using the 300 s bins. (G) The effect of MPEP to decrease firing rate can be accounted for by its reduction of bursting activity. In neurons with a decrease response in firing rate (D, lower panel), the average post-drug firing rates were recalculated after removing the spikes in bursts. The significant decrease in overall firing rate induced by MPEP was no longer evident when spikes that were not in bursts were compared. *P < 0.05 compared with the vehicle group.
group (Fig. 4F; two-way ANOVA with time as repeated measure, $P > 0.05$). However, the combination of the lower doses of MPEP and MK801 significantly increased the number of increase responses (Fig. 4E, $\chi^2 = 111.28, P < 0.001$, versus vehicle + vehicle). A two-way ANOVA with time as repeated measure showed that 3 mg/kg MPEP + 0.01 mg/kg MK801 significantly potentiated increase responses compared with either the vehicle + vehicle [Fig. 4F; treatment, $F(1,97) = 11.45$, $P < 0.001$; time, $F(29,2813) = 14.91, P < 0.001$; treatment $\times$ time interaction, $F(29,2813) = 6.21, P < 0.001$], vehicle + 0.01 mg/kg MK801 [treatment, $F(1,112) = 44.37, P < 0.001$; time, $F(29,3248) = 25.75, P < 0.001$; treatment $\times$ time interaction, $F(29,3248) = 17.58, P < 0.001$] or 3 mg/kg MPEP + vehicle [treatment, $F(1,116) = 40.92, P < 0.001$; time, $F(29,3364) = 25.53, P < 0.001$; treatment $\times$ time interaction, $F(29,3364) = 21.05, P < 0.001$] groups. The combination of 3 mg/kg MPEP and 0.01 mg/kg MK801 treatment also significantly increased the average magnitude [Table 2, one-way ANOVA, $F(3,161) = 13.01, P < 0.001$] and duration [$F(3,161) = 23.05, P < 0.001$] of increase responses compared with either of the antagonists alone or vehicle + vehicle treatment. The low doses of the antagonists alone or combined did not induce any significant change in the temporal profile or magnitude of decrease responses (data not shown).

In groups treated with the higher dose of MK801 (0.1 mg/kg), there was a significant effect on the temporal profile of bursting rate for treatment [Fig. 5A, two-way ANOVA with time as repeated measure, $F(2,289) = 45.56, P < 0.001$] and time [$F(29,8381) = 64.96, P < 0.001$], as well as a significant treatment $\times$ time interaction [$F(58,8381) = 12.87, P < 0.001$]. Both groups treated with the higher dose of MK801 produced a significant decrease in bursting compared with the

### Table 2

Comparison of the average magnitude and duration of significant increase or decrease changes in firing rate in response to different treatments.

<table>
<thead>
<tr>
<th>Group</th>
<th>Increase responses</th>
<th>Decrease responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Magnitude</td>
<td>Duration</td>
</tr>
<tr>
<td>1: Vehicle</td>
<td>1.48 ± 0.07</td>
<td>26.56 ± 5.6</td>
</tr>
<tr>
<td>2: MPEP 10</td>
<td>1.60 ± 0.13</td>
<td>29.3 ± 5.5</td>
</tr>
<tr>
<td>3: Veh + Veh</td>
<td>1.65 ± 0.18</td>
<td>20.6 ± 3.4</td>
</tr>
<tr>
<td>4: Veh + MK 0.1</td>
<td>1.99 ± 0.10</td>
<td>54.3 ± 2.8⁰</td>
</tr>
<tr>
<td>5: MPEP 0.1 + MK 0.1</td>
<td>2.72 ± 0.10⁶</td>
<td>71.7 ± 3.7⁰</td>
</tr>
<tr>
<td>6: Veh + MK 0.01</td>
<td>1.54 ± 0.07</td>
<td>16.3 ± 1.3</td>
</tr>
<tr>
<td>7: MPEP 3 + Veh</td>
<td>1.47 ± 0.05</td>
<td>25 ± 2.8</td>
</tr>
<tr>
<td>8: MPEP 3 + MK 0.01</td>
<td>2.34 ± 0.11⁶</td>
<td>48.4 ± 3.1⁰</td>
</tr>
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Animals received vehicle, MPEP or MK801 as described in Table 1. *$P < 0.05$, compared with the single vehicle group, Student’s t-test. **$P < 0.05$, compared with the vehicle + vehicle group. P* $P < 0.05$, compared with the vehicle + 0.1 mg/kg MK801 group, Bonferroni post-hoc test after one-way ANOVA showed a significant effect. For each variable, separate ANOVAs were run for groups treated with the high doses (groups 3-5) and those treated with the low doses (groups 3-6) of the antagonists. Magnitude is expressed as normalized firing rate and duration is in minutes.

**Figure 3.** MPEP selectively reduces the bursting and firing rates of the more active units. (A, B) Scatter plots of average post-drug firing rate versus baseline firing rate of individual neurons. Line indicates no change from baseline. The distribution of changes in firing rate induced by 10 mg/kg MPEP (A) was different from that of vehicle (B). (C) Neurons in each treatment group were divided into two subsets with lower or higher than median baseline firing rate (1.66 Hz for the total population of recorded neurons). MPEP’s effect on reducing the firing rate was selective for neurons with higher than median baseline rate. *$P < 0.05$ compared with the vehicle group; **$P < 0.05$ compared with the low baseline subgroup in MPEP group. (D-F) The relationship of drug-induced bursting with baseline firing rate. Relative change in bursting was computed by dividing the percentage of spikes in bursts in the post-drug period by the baseline period. Scatter plots for individual neurons are depicted in (D) for the MPEP group and in (E) for the vehicle group. (F) MPEP induced a significantly larger inhibition of bursting in the neurons with higher than median baseline firing rate (>1.66 Hz, see C) than in those with a lower than median baseline rate. *$P < 0.05$ compared with the vehicle group; **$P < 0.05$ compared with the low baseline subgroup in MPEP group.
vehicle + vehicle group [vehicle + 0.1 mg/kg MK801, $F(1,223) = 70.85, P < 0.001$, 10 mg/kg MPEP + 0.1 mg/kg MK801, $F(1,172) = 71.08, P < 0.001$]. However, there was no significant difference between the temporal profiles of burst activity in the 10 mg/kg MPEP + 0.1 mg/kg MK801 group compared with the vehicle + 0.1 mg/kg MK801 group [F(1,183) = 1.65, $P > 0.05$]. In the groups treated with the lower doses of the antagonists, there was a significant effect on the temporal profile of bursting for treatment [Fig. 5B, $F(3,380) = 14.32, P < 0.001$] and time [F(29,11020) = 16.32, $P < 0.001$] and a significant treatment × time interaction [F(87,11020) = 12.68, $P < 0.001$]. The combination of 3 mg/kg MPEP + 0.01 mg/kg MK had a significant effect compared with the vehicle + vehicle group [F(1,213) = 35.93, $P < 0.001$].
vehicle + 0.01 mg/kg MK801 group \([F(1,191) = 14.55, P < 0.001]\) or 3 mg/kg MPEP + vehicle group \([F(1,190) = 7.88, P < 0.001]\). There was a significant effect of treatment on percentage of spikes in bursts [Fig. 5C, one-way ANOVA, \(F(5,562) = 30.86, P < 0.001\)], number of spikes per burst [Fig. 5D, \(F(5,562) = 25.24, P < 0.001\)] and number of bursts per min \([F(5,562) = 43.71, P < 0.001, \text{not shown}\)]. In summary, post-hoc analysis revealed that MPEP, at 10 mg/kg, enhances the effect of 0.1 mg/kg MK801 on the number of bursts and percentage of spikes in bursts but not on the number of spikes per burst. MPEP, at 3 mg/kg, significantly decreased all three measures of bursting, while MK801 at 0.01 mg/kg only decreased the number of spikes per burst. Combination of these lower doses of the two antagonists led to a stronger inhibition of burst activity that was significantly different from the effects of the two antagonists alone.

**The Variability of Firing Rate**

To assess the changes in the variability of firing rate, we calculated the average coefficient of variation (CV) during separate 30 min epochs for each single unit and compared the temporal changes in variability throughout the recording session. Since gradual changes in mean firing rate influence the CV, we also calculated the local measure of variability (CV2, a measure independent of gradual changes in firing rate; see Materials and Methods) for each epoch. The results of the analysis for both measures were similar, though the values for CV2 were generally lower than CV. In the vehicle + vehicle group, there were no significant changes in either CV or CV2, compared with baseline values (Fig. 6A,C). Both values were >1, which indicates a Poisson distribution of ISIs (Holt et al. 1996). Analysis of the groups treated with the high-dose antagonists, using a two-way ANOVA with treatment and epoch as factors, showed a significant effect for treatment \([CV, F(3,1815) = 45.25, P < 0.001; CV2, F(3,1815) = 84.60, P < 0.001]\] and epoch \([CV, F(4,1815) = 2.95, P = 0.01; CV2, F(4,1815) = 2.86, P < 0.05]\] and a significant treatment × epoch interaction \([CV, F(12,1815) = 4.24, P < 0.001; CV2, F(12,1815) = 3.54, P < 0.001]\). At 0.1 mg/kg, MK801 produced a significant decrease in both measures of variability. At 10 mg/kg, MPEP by itself did not significantly influence variability, but the combination of 10 mg/kg MPEP + 0.1 mg/kg MK801 significantly decreased variability [two-way ANOVA between two groups, CV, treatment, \(F(1,915) = 9.78, P < 0.01, \text{epoch}, F(4,915) = 14.26, P < 0.001, \text{treatment} \times \text{epoch interaction}, F(4,915) = 4.36, P < 0.01; CV2, treatment, \(F(1,915) = 18.53, P < 0.001, \text{epoch}, F(4,915) = 5.96, P < 0.001, \text{treatment} \times \text{epoch interaction}, F(4,915) = 3.25, P = 0.01\)]. The combination of the lower doses of both antagonists also led to a significant decrease in both variability measures [Fig. 6B, CV, treatment, \(F(3,1900) = 87.33, P < 0.001, \text{epoch}, F(4,1900) = 2.40, P < 0.05; \text{treatment} \times \text{epoch interaction}, F(12,1900) = 2.18, P = 0.005; \text{Fig. 6D}, CV2, treatment, \(F(3,1900) = 82.38, P < 0.01, \text{epoch}, F(4,1900) = 3.41, P < 0.005; \text{treatment} \times \text{epoch interaction}, \text{CV2} \times \text{epoch interaction}, F(4,1900) = 3.41, P < 0.005). For both CV and CV2, \(P < 0.05)\] compared with the corresponding vehicle + vehicle group; \(*P < 0.05\) compared with the group treated with corresponding dose of MK801 + vehicle. 

![Figure 5. MPEP potentiates the effects of MK801 on spontaneous burst activity of mPFC units. (A, B) Comparison of the temporal profiles of changes in bursting rate for all neurons in each group (details as in Fig. 3D). MK801 (0.1 mg/kg) alone or combined with the higher dose of 10 mg/kg MPEP significantly reduced the bursting activity of mPFC neurons (A). The combination of the lower doses of MPEP (3 mg/kg) and MK801 (0.01 mg/kg) also produced a significant decrease in bursting rate (B). The effects of various treatments on the percentage of spikes in bursts (C) and the average number of spikes per burst (D) were compared following a second injection (MK801 or vehicle). *P < 0.05 compared with the corresponding vehicle + vehicle group; #P < 0.05 compared with the group treated with corresponding dose of MK801 + vehicle.](http://cercor.oxfordjournals.org/DownloadedFromByGuestOnApril30,2016)
F(12,1900) = 1.75, P < 0.05]. There was no significant effect for either of the low doses of the antagonists alone. Thus, the combination of the lower doses of MPEP and MK801 produces a similar effect on variability of firing rate as the high dose of MK801.

**Spontaneous Behavioral Stereotypy**

Previous behavioral studies had shown synergistic behavioral interactions between NMDA and mGlu5 receptor antagonists (Henry et al., 2002; Kinney et al., 2003; Campbell et al., 2004; Homayoun et al., 2004b). To ensure that the present data and the doses used are relevant to these behavioral studies, we measured spontaneous behavioral stereotypy during recording. Vehicle, MPEP (3 or 10 mg/kg) or the low-dose MK801 (0.01 mg/kg) did not produce behavioral stereotypy during the recording session (Fig. 7A, B). As expected, MK801 at 0.1 mg/kg produced behavioral stereotypy characterized by ambulation, rearing, head wag, and oral movements [F(29,116) = 6.01, P < 0.001]. The combination of the lower doses of MPEP and MK801 produced a significant but transient increase in stereotypy score [F(29,87) = 2.85, P < 0.001]. The additive effect of MPEP plus MK801 was more pronounced at the higher doses where pretreatment with MPEP significantly increased the magnitude of behavioral stereotypy after 0.1 mg/kg MK801 [two-way ANOVA with time as repeated measure, treatment, F(1,7) = 2.58, P < 0.05, time, F(29,203) = 15.59, P < 0.001, treatment x time interaction, F(29,203) = 3.20, P < 0.01; post-hoc one-way ANOVA with time as repeated measure, F(29,87) = 9.65, P < 0.001]. To assess whether this behavioral potentiation is correlated with the increased mPFC firing rate, we calculated the coefficients of correlation between the stereotypy score and the average firing rate of the neurons that displayed an increase firing response. For each animal, the stereotypy score for each 5min bin was matched with the average firing rate during the same period. As shown in Figure 7C, there was a poor correlation between stereotypy score and increased mPFC firing activity in the vehicle + vehicle group and
the other groups with low stereotypy behavior. In contrast, there was a high correlation in the vehicle + 0.1 mg/kg MK801 group, as well as in the 10 mg/kg MPEP + 0.1 mg/kg MK801 group. In the 3 mg/kg MPEP + 0.01 mg/kg MK801 group there was a low correlation because the rise in the stereotypy score was transient whereas the firing rate increase was persistent.

Discussion

Modulation of PFC Neuronal Firing by mGlu5 Receptors

Inhibition of mGlu5 receptors decreased the firing rate of approximately half of the PFC neurons. This effect was mostly due to a decrease in bursting activity (defined as periods of non-Poisson high frequency firing) and was dependent on the baseline firing rate of individual neurons. While a phasic influence of mGlu5 receptors on neuronal activity, i.e. the ability of mGlu5 agonists to produce EPSPs (Fitzjohn et al., 1999; Awad et al., 2000), has been demonstrated in some in vitro preparations, the present findings demonstrate that in awake animals mGlu5 receptors exert a profound rate-dependent influence on spontaneous burst activity of cortical neurons. This was a robust influence because it was observed in nearly half of the PFC neurons, suggesting that activation of mGlu5 receptors may be critical for proper functioning of the PFC.

Because systemic route of drug administration was used in this study, the observed effects of mGlu5 receptor antagonist may involve both direct effects in the PFC as well as indirect mechanisms involving areas such as hippocampus and amygdala that are rich in mGlu5 receptors and heavily project to PFC. In either case, the observation of profound changes in PFC spontaneous activity has implications for cognitive and other behavior effects of mGlu5 receptor ligands.

The PFC is required for working memory and cognitive flexibility (Funahashi et al., 1993; Goldman-Rakic, 1996; Bechara et al., 1998; Stefani et al., 2003). While most previous studies have concentrated on the role of dopamine and ionotropic glutamate receptors on these functions (Brozoski et al., 1979; Stefani et al., 2003), recent behavioral studies have described a pivotal role for mGlu5 receptors in PFC-dependent cognitive functions, including working memory (Homayoun et al., 2004b; Naie and Manahan-Vaughan, 2004). The present data demonstrating a rate-dependent regulation of spontaneous bursting in PFC by mGlu5 receptors suggest a possible mechanism for these
behavioral effects. Specifically, in many brain regions neural bursting is considered crucial for transmission of functionally relevant information (Lisman, 1997; Izhikevich et al., 2003). Corticolimbic bursting has been suggested as a critical means for active representation of cognitive goals (Miller and Cohen, 2001) and potential reward (Cooper, 2002). Thus, the regulatory effects of mGlu5 receptors on mPFC bursting may be linked to their role in cognition.

**Interaction between mGlu5 and NMDA Receptors**

The present data demonstrate that mGlu5 and NMDA receptors synergistically regulate PFC firing activity. The functional interaction between mGlu5 and NMDA receptors is of considerable interest because NMDA receptors have been implicated in most cognitive functions, including learning and memory (Riedel et al., 2003), and in many brain disorders, including schizophrenia (Geyer and Moghaddam, 2002). The dose-dependent interaction of the two receptor antagonists on cortical firing observed here closely resembles their interaction in impairing working memory and instrumental learning (Homayoun et al., 2004b). A notable difference between the effects of the two antagonists was that the mGlu5 receptor blockade did not increase firing of single spikes as seen by the NMDA antagonist, but it significantly enhanced the effect of this antagonist. This is consistent with behavioral studies showing that while MPEP by itself does not produce stereotypy or hyperlocomotion, it significantly augments the effect of NMDA antagonism on these behaviors (Henry et al., 2002; Homayoun et al., 2004b). These motor behaviors of NMDA antagonists are thought to be relevant to the psychiatric disorders such as schizophrenia, which are associated with stereotyped tendencies (Carlsson et al., 1993). In the context of the present findings, it is important to emphasize that while rodent motor stereotypy is not considered a ‘cognitive’ behavior, NMDA receptor antagonist-induced motor stereotypes are dependent on the functional integrity of the PFC (Takahata and Moghaddam, 2003). Thus, while future recordings during performance of PFC-dependent cognitive tasks are needed to correlate PFC neuronal activity with disruption of cognitive functioning by mGlu5 and NMDA receptor antagonists, the present findings do provide preliminary evidence that the activity of PFC neurons is relevant to the synergistic behavioral effects of these antagonists.

The mGlu5 receptors enhance NMDA-evoked depolarization in many forebrain regions (Doherty et al., 1997; Pisani et al., 1997; Awad et al., 2000; Mannaiomi et al., 2001; Collett and Collingridge, 2004), and interact with NMDA receptors through shared signaling pathways, including Gq and Homer proteins (Ango et al., 2001; Swanson et al., 2001). Furthermore, the mGlu5 receptors facilitate NMDA-dependent synaptic plasticity through long-term potentiation (Lu et al., 1997; Fendt and Schmid, 2002; Naie and Manahan-Vaughan, 2004). Concomitant attenuation of these post-synaptic mechanisms may be responsible for the interactions between mGlu5 and NMDA receptor antagonists. Moreover, considering the well-described role of NMDA receptors in burst production (Connors and Gutnick, 1990; Kobayashi et al., 1993), it is likely that the effects of MPEP on cortical bursting may be a result of its attenuation of NMDA currents (Mannaiomi et al., 2001; Lea et al., 2002). Taken together, a significant reduction of bursting and an exacerbation of excessive single spike firing may underlie the reported potentiation of the NMDA receptor antagonist effects by MPEP.

Despite relative stability of baseline firing and bursting rates, the pattern of spike firing of cortical neurons is quite variable due to rapid changes in consecutive ISIs. Cortical discharge variability (CV) is significantly higher in vivo than in vitro (Holt et al., 1996) and may increase during periods of functional significance (Softky and Koch, 1993; Baddeley et al., 1997; Lee et al., 1998), including a specific increase in the variability of PFC neuronal discharge during mnemonic activity in monkeys (Compote et al., 2003). The measure CV2 provides a more reliable indicator of changes in variability for the current data because it is insensitive to gradual changes in mean firing rate (Holt et al., 1996). MK801 significantly decreased both CV and CV2, an effect replicated by the combination of the low doses of the two antagonists. These findings suggest that NMDA receptor blockade alters the temporal pattern of spiking in a way that may reduce the flexibility of mPFC neuronal responses. Although mGlu5 receptors do not seem to be involved in this process, they potentiate the effect of NMDA receptor blockade on cortical discharge variability.

**Clinical Implications**

Recent genetic linkage studies in schizophrenia have identified the gene encoding for RGS4, a protein that impacts mGlu5 receptor-mediated activation of the G-protein Gq (Saugstad et al., 1998), as a schizophrenia susceptibility gene (Chowdari et al., 2002). Our finding that mGlu5 receptors control the pattern of spontaneous activity of PFC neurons suggests that dysregulation of mGlu5 receptor-mediated signal transduction, which may occur in schizophrenia, would have a detrimental impact on PFC related functions. This is significant in the context of schizophrenia because post-mortem, imaging and psychological studies have consistently shown that the PFC is a critical site of pathology in this disease (Weinberger et al., 1986; Lewis, 1995; Goldman-Rakic and Selemon, 1997). In addition, other genetic linkage studies now support a link between the NMDA receptor dysfunction and schizophrenia (for reviews, see Harrison and Owen, 2003; Moghaddam, 2003). The present findings on the synergistic NMDA-mGlu5 receptor interactions complement recent molecular and behavioral studies which suggest that modulation of mGlu5 receptor function may be a promising avenue for treating cognitive deficits of schizophrenia if they arise from NMDA receptor dysregulation (Conn, 2003; Moghaddam, 2004).

Recent studies also implicate mGlu5 receptors in substance abuse (Kenny and Markou, 2004). For example, inhibition of mGlu5 receptor function decreases the reinforcing effects of cocaine, morphine and nicotine (Chiamulera et al., 2001; Popik and Wrobel, 2002; Paterson et al., 2003). Regulation of cortical burst firing by mGlu5 receptors, described here, may contribute to the effects of these receptors on reward circuitry (Cooper, 2002) and suggests that strategies based on modulation of mGlu5 receptors may be useful for normalizing glutamate transmission in addictive disorders (Wolf, 1998). However, the clinical use of mGlu5 receptor antagonists should be treated with caution given their disruptive effects on cortical activity and cognitive functions. Finally, previous studies have shown that mGlu5 receptors facilitate hippocampal bursting and are involved in the induction of persistent bursting activity in epileptiform conditions (Merlin, 2002; Thau et al., 2002;
Stoop et al. (2003), suggesting that the detrimental effects of MPEP on cortical bursting activity observed here may also be linked to its potent anticonvulsant effects (Chapman et al., 2000; Nagaraja et al., 2004).

Notes
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