Dendritic Glutamate-Induced Bursting in the Prefrontal Cortex: Further Characterization and Effects of Phencyclidine

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ABSTRACT

To understand the role of N-methyl-D-aspartate (NMDA) receptors in the prefrontal cortex (PFC) and to investigate how the psychotomimetic drug phencyclidine (PCP) may alter PFC function, we made whole-cell recordings from PFC neurons in rat brain slices. Our result showed that most deep layer pyramidal neurons in the PFC were regular spiking cells. They could fire repetitive bursts, however, when activated by glutamate focally applied to the apical dendrite. Application of NMDA to the same dendritic spot also induced bursting, whereas application of \(-\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) evoked single spikes only. Coapplication of AMPA with NMDA evoked more single spikes and decreased NMDA-induced bursting. Experiments with NMDA and AMPA antagonists further showed that dendritic glutamate (dGlu)-induced bursting required NMDA receptor activation and was enhanced when AMPA receptors were blocked. At subanesthetic concentrations, PCP decreased dGlu-induced bursting and altered the temporal characteristics of the bursts by decreasing spikes per burst and increasing interspike intervals within bursts. The latter two changes were not observed when AMPA receptors were blocked, suggesting that they are secondary to the increased AMPA receptor contribution to glutamate responses evoked in the presence of PCP. These results suggest that NMDA receptors are essential for PFC pyramidal cells to fire in bursts in response to dGlu input and that PCP suppresses dGlu-induced bursting. Since bursting is necessary for pyramidal cells to activate GABA interneurons, the suppression effect of PCP may further lead to a weakening of the connections from pyramidal cells and GABA interneurons, thereby contributing to PCP’s psychotomimetic effects.

Cortical pyramidal neurons are traditionally grouped into two categories: regular spiking and intrinsic bursting cells. When activated by a sustained depolarization induced by intrasomatic current injection, regular spiking cells fire repetitive single spikes or an initial doublet followed by single spikes. Intrinsic bursting cells, on the other hand, fire repetitive bursts. The two types of cells also differ morphologically (Chagnac-Amitai et al., 1990; Mainen and Sejnowski, 1996). Although fewer than regular spiking cells, intrinsic bursting neurons are present in most cortical areas examined and have been suggested to play an important role in synchronized activities in the cortex (Chagnac-Amitai and Connors, 1989).

In a preliminary study, we reported that regular spiking neurons in the prefrontal cortex (PFC) could fire repetitive bursts when they were activated by glutamate focally applied to the apical dendrite (Zhang and Shi, 1999). Dendritic glutamate (dGlu)-induced bursting has also been reported in the sensorimotor cortex (Schwindt and Crill, 1997). Studies in the latter area further suggest that the activity is triggered by activation of voltage-gated Ca\(^{2+}\) channels due to dendritic depolarization (Schwindt and Crill, 1999). In the PFC, however, we found that NMDA but not AMPA mimicked dGlu-induced bursting (Zhang and Shi, 1999), suggesting that the mechanism underlying dGlu-induced bursting in the PFC may differ from that observed in the sensorimotor cortex, depending upon activation of NMDA receptors.

In the present study, experiments were carried out to further test whether dGlu-induced bursting in the PFC is simply due to a dendritic depolarization or whether it requires activation of NMDA receptors. Since both NMDA and non-NMDA receptors are activated by glutamate, this study also asked whether, and how, non-NMDA receptors, especially AMPA receptors, may contribute to dGlu-induced bursting. Finally, we examined the effects of the psychotomimetic drug phencyclidine (PCP) on dGlu-induced bursting because 1) PCP is a potent NMDA receptor channel blocker, 2) PCP is thought to produce part of its psychotomimetic effects by

Abbreviations: PFC, prefrontal cortex; dGlu, dendritic glutamate; NMDA, N-methyl-D-aspartate; AMPA, \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; PCP, phencyclidine; CCD, charge-coupled device; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline; CGP37849, (E)-(\(\pm\))-2-amino-4-methyl-5-phosphono-3-pentenoic acid; TTX, tetrodotoxin; ISI, interspike interval.
affecting PFC function, and 3) changes in bursting would significantly alter information processing in the PFC, thereby contributing to PCP's psychotomimetic effects.

**Materials and Methods**

**Preparation of Slices.** All procedures were performed in accordance with those outlined in the Guide for the Care and Use of Laboratory Animals published by the U.S. Public Health Service and approved by the Yale Animal Care and Use Committee. Male Sprague-Dawley albino rats weighing between 33 and 104 g (2–5 weeks old; Charles River Laboratories, Inc., Wilmington, MA) were used. Brain slices were prepared as described previously (Shi et al., 1997; Zheng et al., 1999). Briefly, rats were anesthetized with chloralhydrate (400 mg/kg i.p.) and decapitated. The brains were quickly dissected and submerged in an ice-cold perfusion medium containing 125 mM NaCl, 3 mM KCl, 1.4 mM MgSO₄, 1.2 mM CaCl₂, 1.2 mM NaH₂PO₄, 25 mM NaHCO₃, 10 mM glucose, 10 mM sucrose, and saturated with 95% O₂/5% CO₂. A block of tissue containing the PFC was cut and glued on the cutting stage of a Vibratome (OTTO-4000 tissue slicer; FHIC Inc., Bowdoinham, ME). Serial coronal slices (280–300 μm) were cut and transferred to an incubating chamber where they were held at 35°C for at least 1 h before recording.

**Electrophysiological Recording.** During recording, slices were continuously perfused at room temperature in a submerged recording chamber placed on the fixed stage of an upright microscope (BX50WI; Olympus Optical Co. Ltd., Tokyo, Japan). Individual neurons were visualized using Nomarski optics and a 40X long working distance, water immersion objective (3 mm, NA 0.7; Olympus). The neurons were visualized using Nomarski optics and a 40X long working distance, water immersion objective (3 mm, NA 0.7; Olympus). The images were enhanced with an infrared-sensitive CCD camera (CCD-300T-RC; Dage-MTI, Michigan City, IN; infrared blocking filter removed) and displayed on a video monitor. Pyramidal cells were identified based on their pyramidal shape and the presence of the apical dendrite. Whole-cell recordings were made from the soma identified based on their pyramidal shape and the presence of the apical dendrite. Whole-cell recordings were made from the soma using electrodes pulled from thin-walled glass capillaries (o.d. = 1.5 mm; WPI, Sarasota, FL). Electrodes were filled with a solution containing 140 mM potassium gluconate, 0.1 mM CaCl₂, 2 mM MgSO₄, 1 mM EGTA, 2 mM ATP₂, 0.1 mM GTPNa₃, 10 mM HEPES, and 0.5% biocytin, pH 7.25, and had a resistance of 7 to 12 MΩ. Voltage and current signals were recorded with an Axoclamp-2A (Axon Instruments, Inc., Union City, CA) interfaced to a personal computer (Dimension XPS Pro200m; Dell, Austin, TX). Data were digitized (Digidata 1200; Axon) and stored on disks using pClamp (v.3.4; J. Santos-Sacchi, Yale University) or pClamp (v.8; Axon). Off-line data analysis was performed using pClamp and Visual Basic Macros in Microsoft Excel. All potentials reported were corrected for junction potential (15 mV) between the electrode and the perfusion medium, which was calculated based on ionic compositions of the intra- and extracellular solutions using pClamp. A similar junction potential was reported by previous studies using similar solutions (e.g., Neher, 1992).

**Drug Application.** Glutamate and its agonists were applied focally to the apical dendrite (50–150 μm away from the soma) using multibarrel micro-iontophoretic electrodes. The tip of the electrode was 2 to 3 μm in diameter. One barrel was filled with 0.5 M NaCl and used as the balance electrode. Current through the balance electrode was automatically adjusted so that the sum of currents through all barrels equaled zero. Other barrels were filled with solutions containing glutamate (100 mM, pH 8.5), NMDA (100 mM, pH 8.5), and AMPA (100 mM, pH 8.5), respectively. A positive retaining current (10 nA) was applied to all drug barrels to eliminate passive diffusion when the drug was not ejected. All drugs were ejected with negative currents ranging from 0 to 50 nA. In some experiments, glutamate and its agonists (50–100 μM) were applied using pressure ejection (5–40 psi for 10–20 ms at 15–40 pulses/s). The pipette used for pressure ejection had the same characteristics as the pipette used for recording. Increasing amounts of glutamate were applied by increasing the frequency of the ejection (pulses per second). Results obtained with the two application methods were similar and were, therefore, analyzed together. All other drugs were administered through the bath via a three-way stopcock.

Drugs used in this study and their sources are glutamate (Sigma-Aldrich, St. Louis, MO); NMDA, AMPA, NBQX, phencyclidine, and ketamine (Sigma/RBI, Natick, MA); CGP37849 (Ciba Specialty Chemicals, Basel, Switzerland); and tetrodotoxin (TTX; Alomone Labs Ltd., Jerusalem, Israel).

**Biocytin Staining.** At the end of recording, slices were fixed overnight with 4% paraformaldehyde in phosphate buffer solution (pH 7.4) and then washed and transferred to phosphate buffer solution containing 0.5% Triton X-100 and 1% hydrogen peroxide for 3 h. After rinsing, slices were incubated with avidin-biotinylated peroxidase complex (Vectorstain Elite ABC kits PK-6100; Vector Laboratories Inc., Burlingame, CA) in the presence of 0.5% Triton X-100 for 36 h. The peroxidase was visualized by reacting the slice with diaminobenzidine and hydrogen peroxide.

**Statistics.** The statistical significance of a drug effect on dGlu-induced bursting was determined by comparing dGlu-induced bursting before and after the drug application using analysis of covariance. The covariate was the response of the cell prior to drug perfusion. All numerical data were expressed as mean ± S.E.M.

**Results**

**Firing Properties of PFC Pyramidal Cells.** Data presented in this study were obtained from 280 PFC neurons; all were visually identified as pyramidal cells using infrared videomicroscopy (see Materials and Methods). After biocytin staining, 181 cells were recovered, and all were confirmed to be pyramidal cells. An example of an intracellally stained pyramidal cell is shown in Fig. 1A. Most labeled cells were located in layers V and VI of the prelimbic subdivision of the PFC (Fig. 1B). Some were found in the adjacent infralimbic cortex. No difference was observed between the results obtained from the two areas.

All cells were quiescent at rest, with a resting potential ranging from −71 to −83 mV (−76.6 ± 0.2 mV; n = 242). When depolarized by intrasomatic current steps (20–450 pA for 1500 or 3500 ms), all exhibited repetitive firing. Most cells showed an initial doublet (n = 164) or a triplet (n = 2) followed by single spikes (Fig. 2A). The remaining cells (n = 53) fired repetitive single spikes only. Repetitive bursting was not observed in any of the cells examined.

Previous studies suggest that the ability of a neuron to fire in bursts is influenced by the membrane potential. In the thalamus, for example, burst firing is observed only when the cell is activated from a hyperpolarized state (Jahnsen and Llinas, 1984). To test whether this is also the case for PFC...
neurons (Zhang and Shi, 1999), we showed that dGlu could evoke repetitive bursting. To confirm the finding, techniques were used to record from cells in the somatosensory cortex. In 22 pyramidal cells recorded in layers V and VI, 6 exhibited repetitive doublets or triplets when depolarized by intrasomatic current steps (Fig. 2B). The remaining cells showed either single-spike firing (n = 9) or an initial doublet followed by single spikes (n = 7). The resting potential of these cells (76.8 ± 4.9 mV; n = 22) was similar to that of PFC neurons (−76.6 ± 0.2 mV; n = 242). These results suggest that the PFC differs from other cortical areas and lacks intrinsic bursting cells.

**dGlu-Induced Bursting.** In a preliminary study (Zhang and Shi, 1999), we showed that dGlu could evoke repetitive bursting in PFC pyramidal cells. To confirm the finding, glutamate was focally applied to the apical dendrite in 122 PFC pyramidal cells. Of these cells, 78 exhibited repetitive bursting and 44 showed regular spiking. However, when cells of the latter group were hyperpolarized (2–20 mV from the resting potential), all showed repetitive bursting in response to dGlu application (n = 34). The minimum hyperpolarization required for bursting was not systemically determined because it varied from cell to cell and depended on both the amount and the site of glutamate application. The latter may reflect the fact that glutamate receptors are unevenly distributed on the apical dendrite (Dodt et al., 1998).

Figure 3 shows recordings from two typical PFC pyramidal cells. One fired repetitive bursts at rest in responding to dGlu application (Fig. 3A), and one required a small hyperpolarization to show similar responses (Fig. 3B). Note that at currents below the threshold for spiking, dGlu induced a smooth depolarization with no signs of oscillatory activity. Once the ejection current reached the threshold, the cell began to oscillate rhythmically. The oscillation, in turn, triggered repetitive bursting. Typically, each burst consisted of two to four spikes with ISIs ranging from 12 to 133 ms. Burst frequency increased with increasing glutamate. However, at high ejection currents, bursts were often followed by a train of single spikes.

**Effects of NMDA and AMPA.** To study the role of NMDA and AMPA receptors in dGlu-induced bursting, we have previously compared effects of glutamate, NMDA, and AMPA (Zhang and Shi, 1999). The comparison was made, however, between cells. In this study, we compared the three agonists in the same cell by applying them to the same dendritic sites using multibarrel iontophoretic electrodes. Such comparison minimizes possible variance due to differences between cells and drug application sites. In all cells studied (n = 7), the three agonists depolarized the cell and induced firing. However, unlike glutamate and NMDA, both of which induced repetitive bursting (Fig. 4, A and B), AMPA induced single spikes only (Fig. 4C).

Effects of glutamate and NMDA were not, however, entirely identical. Bursts induced by glutamate were often followed by single spikes, especially at high ejection currents (Fig. 4A). NMDA, on the other hand, induced bursts only; no single spikes were observed, even at the highest ejection current (Fig. 4B). Glutamate-induced bursts also consisted of fewer spikes and had longer within-burst ISIs than those
induced by NMDA (Fig. 4, A and B). To quantitatively compare their effects, spikes per burst and within-burst ISIs were determined. For simplicity, only the number of spikes in the first evoked burst and the first ISI within the first burst (ISI1) were measured. On average, bursts induced by glutamate consisted of 2.28 ± 0.18 spikes, whereas those induced by NMDA consisted of 5.14 ± 0.77 spikes (F1,11 = 18.46, p < 0.001). The average ISI1 was 28.8 ± 8.2 ms for glutamate-induced bursts and 15.4 ± 3.1 ms for NMDA-induced bursts (F1,11 = 9.02, p < 0.05). These differences suggest that activation of non-NMDA receptor by glutamate may attenuate glutamate’s ability to induce bursting through NMDA receptors.

NMDA-AMPA Interaction. To more directly test whether coactivation of AMPA receptors decreases the ability of NMDA to induce bursting, AMPA was coapplied with NMDA using multibarrel electrodes. In all 12 cells tested, coadministration of AMPA increased single spikes and decreased bursting induced by NMDA (Fig. 5), resulting in a firing pattern similar to that induced by glutamate. On average, AMPA application decreased spikes per burst induced by NMDA from 5.9 ± 1.3 to 2.6 ± 0.4 and increased ISI1 from 19.5 ± 1.7 to 40.7 ± 5.5 ms (n = 12). Both effects were statistically significant (spikes per burst: F1,11 = 18.89, p < 0.001; ISI1: F1,12 = 29.66, p < 0.001).

Effects of NMDA and AMPA Antagonists. To confirm further that dGlu-induced bursting requires activation of NMDA receptors, glutamate was applied in the presence of the competitive NMDA antagonist CGP37849 or the AMPA antagonist NBQX. As might be expected, dGlu induced single spikes only when NMDA receptors were blocked by CGP37849 (1–10 μM, n = 6; Fig. 6A). However, it reliably induced bursting when AMPA receptors were blocked by NBQX (5–10 μM, n = 4; Fig. 6B).

Comparing the response of the cell before and after NBQX showed that the AMPA antagonist not only did not prevent dGlu from inducing bursting, but further enhanced its ability to induce bursting. Thus, under control conditions, bursts induced by dGlu were often followed by single spikes. Following NBQX, glutamate induced bursts only (Fig. 5B). Bursts induced in the presence of NBQX also consisted of more spikes (increased from 3.2 ± 0.2 to 6.0 ± 0.7 spikes/burst,
F_{1,5} = 11.27, p < 0.05) and had shorter ISIs (decreased from 21 ± 1.7 to 14.5 ± 1.6 ms, F_{1,5} = 7.4, p < 0.05) than those induced under control conditions. Again, these results support the suggestion that AMPA receptor activation is detrimental to burst generation.

Effects of Synaptic Blockade. Our previous study suggests that dGlu-induced bursting is a direct effect of glutamate on the recorded cell because displacement of the iontophoretic electrode a few micrometers away from the apical dendrite resulted in an abrupt reduction in the response of the cell (Zhang and Shi, 1999). To further confirm this suggestion and to reduce possible polysynaptic effects mediated through glutamate and GABA neurons, NMDA was applied to selectively activate NMDA receptors, whereas AMPA and GABA_{A} receptors were blocked by NBQX (5 μM) and picrotoxin (30 μM), respectively. In all 12 cells tested, dendritic NMDA application reliably induced bursting.

To further block synaptic interactions in the slice, the Ca^{2+} channel blocker Cd^{2+} (50–100 μM, n = 6) was added to the medium already containing picrotoxin (30 μM) and NBQX (5 μM). In three cells, Cd^{2+} completely blocked NMDA-induced firing. However, when the NMDA ejection current was increased, these cells were able to fire again in bursts. In the remaining three cells, NMDA-induced bursting persisted in the presence of Cd^{2+}. Bursts evoked in the presence of Cd^{2+} differed, however, from those seen under control conditions (Fig. 7A); spikes per burst were markedly increased (from 2.3 ± 0.2 to 11.2 ± 3.0 spikes/burst, F_{1,9} = 8.4, p < 0.05), whereas within-burst ISIs were decreased (ISI_{1} decreased from 33.7 ± 4.2 to 19.8 ± 1.7 ms, F_{1,9} = 18.9, p < 0.005). Whether these changes are due to inhibition of Ca^{2+}-dependent K^{+} channels remains to be investigated.

In another set of experiments, TTX (0.5 μM) was added to block action potential-dependent synaptic interactions. In all 11 cells examined, TTX completely blocked the generation of action potentials. However, it failed to block membrane oscillations induced by dGlu or NMDA (Fig. 7B).

Effects of PCP. PCP is an NMDA receptor channel blocker. Compared with CGP37849, PCP (1–5 μM) was less effective in inhibiting dGlu-induced bursting. In four of seven cells, CGP37849 (1–10 μM) transformed dGlu-induced bursting into single-spike firing (Fig. 4A). In the three remaining cells, CGP37849 decreased the number of bursts and then completely stopped firing. PCP also decreased the number of bursts but failed to completely convert bursting into single-spike firing. Bursts induced in the presence of PCP differed, however, from those observed under control conditions; spikes per burst were decreased, whereas within-burst ISIs were increased (Fig. 8A). Unexpectedly, a high concentration of PCP (10 μM) decreased the number of bursts without affecting spikes per burst and within-burst ISIs (n = 5; Fig. 9). At this concentration, PCP also produced a hyperpolarization of the cell (4.9 ± 0.7 mV, n = 5).

To quantitatively compare effects produced by different concentrations of PCP, we counted cells showing changes in spikes per burst and within-burst ISIs, and measured ISIs before and after PCP. Because of the slow onset, the effects of PCP were measured 4 to 8 min after PCP perfusion. The results are summarized in Fig. 9.

As shown in Fig. 9A, PCP produced either a decrease or no change in spikes per burst when applied at 1 to 5 μM. At 10 μM, however, PCP increased spikes per burst in 40% of cells. When averaged, changes in spikes per burst induced by 1, 2 to 2.5, and 10 μM PCP were statistically insignificant. The decrease induced by 5 μM PCP, however, was significant (F_{1,9} = 5.29, p < 0.05; Fig. 9B).

Within-burst ISIs were increased in most cells by 1 to 5 μM PCP and decreased by 10 μM PCP (Fig. 9C). On average, ISI_{1}
Discussion

This study shows that most deep-layer pyramidal cells in the PFC fire single spikes or an initial doublet followed by single spikes in response to somatic depolarization. The same cells fire repetitive bursts when activated by glutamate applied to the apical dendrite. dGlu-induced bursting requires NMDA receptor activation and is attenuated when AMPA receptors are coactivated. Activation of AMPA receptors favors single-spike firing. The psychotomimetic drug PCP decreases dGlu-induced bursting and alters the temporal characteristics of the bursts. The latter effect is not observed when AMPA receptors are blocked, suggesting that it is secondary to the increased AMPA contribution to a glutamate-evoked response in the presence of PCP.

Lack of Intrinsic Bursting Cells in the PFC. Cortical pyramidal neurons are traditionally grouped into two categories: regular spiking and intrinsic bursting cells (see the introduction). The present study suggests that the PFC differs from most other cortical areas and lacks intrinsic bursting cells, cells that are capable of firing repetitive bursts upon somatic depolarization. This finding is unlikely to be an artifact of recording since, using the same techniques, we were able to find intrinsic bursting cells in the somatosensory cortex. Furthermore, using traditional intracellular recording techniques, several groups also reported a lack of intrinsic bursting cells in the PFC (Penit-Soria et al., 1987; Foehring et al., 1991; de la Peña and Gejo-Barrientos, 1996;...
Haj-Dahmane and Andrade, 1996; Henze et al., 2000). In one study, however, repetitive bursting was observed in 13% of PFC pyramidal cells (Yang et al., 1996). The cause for the difference is unknown. Although it remains to be confirmed, the lack of intrinsic bursting cells would suggest that bursting activities in the PFC are more dependent on dendritic glutamate input and, thus, more susceptible to the suppression effect of PCP compared with other cortical areas.

dGlutamate-induced Bursting Requires Activation of NMDA Receptors. This study confirms our previous results (Zhang and Shi, 1999), showing that PFC cells could fire repetitive bursts when activated by glutamate applied to the apical dendrite. dGlutamate-induced bursting has been reported previously in the sensorimotor cortex (Schwindt and Crill, 1997) and has been suggested to be mediated by a dendritic depolarization and subsequent activation of voltage-gated Ca^{2+} channels (Schwindt and Crill, 1999). Supporting this suggestion, direct intradendritic current injection evokes or promotes burst firing in hippocampal pyramidal cells (Wong and Stewart, 1992).

The present study suggests, however, that dGlutamate-induced bursting in the PFC requires more than just a depolarization. Dendritic AMPA application clearly depolarized the cell and induced firing. However, unlike glutamate and NMDA, AMPA evoked single spikes only; no repetitive bursting was observed at any AMPA currents tested. By applying glutamate, NMDA, and AMPA to the same dendritic sites in the same cells, this study ruled out the possibility that the observed differences between these agonists are due to different cells sampled or different dendritic sites to which the drugs were applied. Further confirming the role of NMDA receptors in dGlutamate-induced bursting, this study showed that when NMDA receptors were blocked, glutamate, like AMPA, induced single spikes only. Thus, in at least the PFC, dGlutamate-induced bursting requires NMDA receptor activation.

dGlutamate-induced Bursting Is a Direct Effect of Glutamate on the Recorded Cell. Several lines of evidence suggest that dGlutamate-induced bursting is a direct effect of glutamate on the recorded cell. As shown previously, displacement of the iontophoretic electrode a few micrometers away from the apical dendrite resulted in an abrupt reduction in the response (Zhang and Shi, 1999). This study further showed that dGlutamate-induced bursting persisted in the presence of the GABAA antagonist picrotoxin, suggesting that GABA interneurons are unlikely to be involved. Blockade of synaptic transmissions by Cd^{2+} or TTX also failed to prevent dGlutamate from inducing bursting or membrane oscillations.

The ionic mechanism underlying dGlutamate-induced bursting remains to be further investigated. Our results with Cd^{2+} and TTX suggest that both the voltage-gated Ca^{2+} and Na+ channels participate in the initiation of the activity; however, neither is essential. These properties are similar to those of NMDA spikes evoked by glutamate applied to the basal dendrite (Schiller et al., 2000). Whether NMDA spikes are present in the apical dendrite and how they may be related to dGlutamate-induced bursting remain to be investigated.

Effects of PCP. Both PCP and ketamine induced a partial inhibition of dGlutamate-induced bursting. Given the concentrations used, the result seems to contradict the reported affinities of PCP and ketamine to NMDA receptors (e.g., Bresink et al., 1995). One possible explanation is that the affinities were measured in the absence of Mg^{2+}, whereas the present study was performed in the presence of Mg^{2+}. Physiological concentrations of Mg^{2+} are known to decrease the ability of PCP to block NMDA receptor channels (e.g., Lerma et al., 1991).

It is important to point out that the concentrations of PCP used in this study are in the range produced by psychotomimetic doses of PCP (Proksch et al., 2000). Given that, our results would suggest that the psychotomimetic effects of PCP are associated with a partial and not a complete blockade of NMDA receptors. It is perhaps because of this partial blockade that the PFC continues to operate in the presence of PCP. However, changes in bursting induced by PCP may significantly alter the way information is processed within the PFC.

Dual cell recordings show that synaptic connections between pyramidal cells have relatively high release probability and, therefore, are most efficient in transferring signals carried by single spikes (Thomson, 1997; Markram et al., 1998; see also review by Thomson, 2000). In contrast, slow single-spike firing generates little or no response at connections from pyramidal cells to interneurons. For reliable transmission, the presynaptic pyramidal neuron must fire in bursts (see review by Thomson, 2000). Furthermore, the shorter the within-burst ISIs, the larger the postsynaptic response in a GABA interneuron. The response also increases with increasing spikes per burst. If these findings hold true in the PFC, the observed effects of PCP would lead to a weakening of the connections from pyramidal cells to GABA interneurons.

Unexpectedly, PCP at a high concentration (10 μM) produces no significant effect on within-burst ISIs and spikes per burst. It is possible that changes in bursting mediated through NMDA receptors are reversed by PCP’s effects on non-NMDA sites (Johnson and Jones, 1990). Although further studies are needed to test this and other possibilities, preliminary experiments suggest that the membrane hyperpolarization produced by the high concentration of PCP is partially responsible for the unexpected result. In those experiments, reversal of the hyperpolarization by current injection restored the ability of PCP to decrease spikes per burst and to increase within-burst ISIs (W.-X. Shi, unpublished observation).

Role of AMPA Receptors in dGlutamate-induced Bursting and in the Effects of PCP. Although dGlutamate-induced bursting does not require AMPA receptor activation, this study suggests that AMPA receptors play an important modulatory role in the activity. Several lines of evidence suggest that activation of AMPA receptors favors single-spike firing and decreases the bursting mediated by NMDA receptors. Thus, unlike NMDA, which evoked bursts only, glutamate often induced a mixture of bursts and single spikes. When AMPA receptors were blocked, however, glutamate induced bursts only, suggesting that AMPA receptor activation is responsible for the generation of single spikes. Further supporting this suggestion, coapplication of AMPA with NMDA reduced NMDA-induced bursting and, at the same time, increased single-spike firing.

AMPA receptors may also play a role in PCP-induced changes in spikes per burst and within-burst ISIs. This study showed that when AMPA receptors were blocked, PCP produced no effects on the two measurements. This result, together with the finding that AMPA receptor coactivation
decreases NMDA-induced bursting, suggests that changes in spikes per burst and within-burst ISIs induced by PCP are secondary to the increased AMPA receptor contribution to glutamate responses evoked in the presence of PCP.

The modulatory role of AMPA receptors may provide part of the explanation for why an AMPA antagonist can reverse some of the behavioral effects induced by PCP (Moghaddam and Adams, 1998; Mathe et al., 1999). When administered systemically, PCP is known to increase PFC glutamate release (Moghaddam and Adams, 1998). In the presence of PCP, this increase would preferentially activate AMPA receptors, thus further impairing the ability of glutamate to induce bursting. By removing the AMPA-mediated detrimental effect on burst generation, an AMPA antagonist would enable dGlu to regain its ability to induce bursting, thereby reversing some of the behavioral effects of PCP.

In summary, this study shows that NMDA receptors are essential for dGlu to induce burst firing in PFC pyramidal cells and that PCP suppresses the activity. Given the importance of bursting, the observed effects of PCP may significantly alter information processing in the PFC and, thus, contribute to PCP’s psychotomimetic effects.

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