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Presynaptic effects of NMDA receptors enhance parvalbumin cell-mediated inhibition of pyramidal cells in mouse prefrontal cortex

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Abstract

Background—Testing hypotheses regarding the role of N-methyl-D-aspartate receptor (NMDAR) hypofunction in schizophrenia requires understanding the mechanisms of NMDAR regulation of prefrontal cortex (PFC) circuit function. NMDAR antagonists are thought to produce pyramidal cell (PC) disinhibition. However, inhibitory parvalbumin-positive basket cells (PVBCs) have modest NMDAR-mediated excitatory drive, and thus are unlikely to participate in NMDAR antagonist-mediated disinhibition. Interestingly, recent studies demonstrated that presynaptic NMDARs enhance transmitter release at central synapses. Thus, if presynaptic NMDARs enhance GABA release at PVBC-to-PC synapses, they could participate in NMDAR-dependent PC disinhibition. Here, we examined if presynaptic NMDAR effects could modulate GABA release at PVBC-to-BC synapses in mouse PFC.

Methods—Using whole-cell recordings from synaptically-connected pairs in mouse PFC, we determined if NMDA or NMDAR antagonist application affects PVBC-to-PC inhibition in a manner consistent with a presynaptic mechanism.

Results—NMDAR activation enhanced by ~40% the synaptic current at PVBC-to-PC pairs. This effect was consistent with a presynaptic mechanism, since it was 1) observed with postsynaptic NMDARs blocked by intracellular MK801, 2) associated with lower rate of transmission failures and higher transmitter release probability, and 3) blocked by intracellular MK801 in the PVBC. NMDAR antagonist application did not affect the synaptic currents in PVBC-to-PC pairs, but reduced the inhibitory currents elicited in PCs with simultaneous glutamate release by extracellular stimulation.

Conclusions—We demonstrate that NMDAR activation enhances PVBC-to-PC inhibition in a manner consistent with presynaptic mechanisms, and suggest that the functional impact of this presynaptic effect depends on the activity state of the PFC network.

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Disclosures

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Keywords

parvalbumin; NMDA; inhibition; prefrontal cortex; basket cell; pyramidal neuron

Introduction

NMDA receptor (NMDAR) antagonists induce in healthy subjects behavioral alterations resembling core clinical features of schizophrenia (1, 2), and exacerbate these features in individuals with the illness (3). These observations suggest that the disease process involves NMDAR hypofunction (4). Understanding the mechanisms by which NMDARs regulate cortical circuit function is therefore crucial for testing hypotheses that NMDAR hypofunction is involved in the pathophysiology of schizophrenia.

NMDARs are prominent at excitatory synapses onto cortical pyramidal cells (PCs). Paradoxically, systemic NMDAR antagonist administration to rats increases PC activity in the prefrontal cortex (PFC), while reducing the firing of putative inhibitory neurons (5). These data led to the interpretation that inhibitory interneurons have stronger NMDAR-mediated excitatory drive than PCs, and therefore NMDAR antagonism causes PC disinhibition (5, 6).

Parvalbumin-positive (PV⁺) interneurons are a major source of inhibitory inputs on PCs (7), and PV⁺ neuron alterations may contribute to PFC microcircuit dysfunction in schizophrenia (8–10). Moreover, it has been proposed that NMDAR hypofunction at glutamate synapses onto PV⁺ neurons could decrease PV⁺ neuron activity, disinhibiting the PCs (6). However, NMDAR-mediated excitatory postsynaptic currents are quite small in PV⁺ neurons compared to PCs or other interneuron subtypes (11–22). Consistent with weak NMDAR-mediated drive, the synaptic activation of PV⁺ neurons is largely NMDAR-independent (15, 23). Therefore, it seems unlikely that NMDAR antagonists cause PC disinhibition by decreasing PV⁺ neuron activity.

Interestingly, recent studies show that, as originally revealed in the spinal cord (24), presynaptic NMDARs control transmitter release at cortical synapses, suggesting novel mechanisms of NMDAR action in cortical circuits. For example, presynaptic NMDARs enhance glutamate release at excitatory synapses onto hippocampal (25) and neocortical (26, 27) PCs, but not at excitatory synapses onto PV⁺ neurons (26). Moreover, NMDARs might regulate GABA release in neocortex (28, 29), since presynaptic NMDAR subunits were localized by electron microscopy at neocortical inhibitory synapses (30, 31). Furthermore, NMDAR activation increases synaptic inhibition onto PCs, possibly via presynaptic mechanisms.

If NMDARs enhance GABA release at inhibitory PV⁺-to-PC synapses, then NMDAR antagonists could disinhibit PCs by interfering with such a mechanism. However, it is currently unknown if NMDARs regulate the strength of PV⁺-to-PC synaptic transmission in PFC. Thus, we studied neurotransmission between presynaptic PV⁺ basket cells (PVBCs) and postsynaptic PCs in mouse PFC to test the hypothesis that NMDAR stimulation presynaptically enhances PVBC-mediated inhibition of PCs. Consistent with this hypothesis,

our data suggest that NMDAR activation enhances the strength of PVBC-to-PC inhibition, apparently via presynaptic effects. Moreover, we suggest that NMDAR antagonists, or NMDAR hypofunction in schizophrenia, might affect PVBC-to-PC inhibition in specific network activity states.

Methods and Materials

Animals and slice preparation

Experiments were performed in male and female G42 mice (Jackson Laboratory), which express green fluorescent protein (GFP) exclusively in PV⁺ neurons (32). Animals were deeply anaesthetized with isoflurane following NIH guidelines, as approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Coronal brain slices (300 μ m-thick) were prepared from the frontal cortex of 27–66-day-old mice (mean: 39 days) using methods described previously (33), and incubated in artificial cerebrospinal fluid (ACSF), containing (mM): NaCl, 125; KCl, 2.5; Na₂HPO₄, 1.25; glucose, 10; NaHCO₃, 25; ascorbate, 0.4; MgCl₂, 1; CaCl₂, 2, pH 7.3–7.4, at room temperature for >30 min before recording (33).

Electrophysiological recordings and data analysis

Slices were superfused in a submersion chamber at 2 ml/min with oxygenated ACSF at 30–32°C. Whole-cell recordings were obtained from PCs and PV⁺ neurons targeted in layer 3 of the infralimbic, prelimbic or anterior cingulate regions of the medial PFC using Olympus or Zeiss microscopes with infrared differential interference contrast, epifluorescence illumination and CCD cameras (EXiAqua, Q-Imaging)(33). For PV⁺ neuron recordings, borosilicate glass pipettes (resistance: 3–6 M Ω) were filled with (mM): KGluconate, 120; KCl, 10; HEPES, 10; EGTA, 0.2; MgATP, 4.5; NaGTP, 0.3; NaPhosphocreatine, 14, containing 0.4–0.5 % biocytin, to identify the morphology post-hoc (34). For PC recordings, the pipettes were filled with (mM): KGluconate, 60; KCl, 70; HEPES, 10; EGTA, 0.2; MgATP, 4.5; NaGTP, 0.3; NaPhosphocreatine, 14; MK801, 1. This solution increases the GABA_A current driving force, facilitating inhibitory postsynaptic current (IPSC) detection, and blocks NMDARs intracellularly. In Figure 3, MK801 (1 mM) was added to the solution used to patch the PV⁺ neurons.

Recordings were obtained using Multiclamp 700B amplifiers (Molecular Devices), low-pass filtered (4–6 kHz), and digitized (10 or 20 kHz) using Power 1401 interfaces and Signal 5 software (Cambridge Electronic Design). In voltage-clamp, the series resistance was continuously monitored via the response to 5 mV voltage steps. Only recordings with series resistance <15 M Ω that changed less than 15% were used for analysis. In current-clamp, series resistance and pipette capacitance were cancelled using bridge and capacitance neutralization. Cells included in this study had resting membrane potential between –60 and –80mV.

To identify PV⁺-to-PC connected pairs, action potentials were elicited in the PV⁺ neuron with suprathreshold current pulses (2–3 ms, 2 nA) at 0.2 Hz, while searching for an IPSC in

the PC (33). In connected pairs, the PV⁺ cells were stimulated at 0.13 Hz, recording baseline IPSCs for 10 min, followed by 15 min application of 10 μ M NMDA or 100 μ M D,L-AP5.

To estimate changes in IPSC amplitude, 30–40 traces were averaged (including failures) and the IPSC amplitude was measured as the difference between the IPSC peak and baseline current 5–3 ms preceding the IPSC. To analyze transmission failures, the recordings were low pass-filtered digitally at 1 kHz. A trial was considered a failure if the peak current at the time of the average IPSC peak was <3 times the S.D. of the baseline current (35).

To estimate changes in the quantal parameters of transmission, we applied multiple probability fluctuation analysis (36) to IPSC trains. Briefly, the mean peak IPSC amplitude ($\bar{I}$) and variance (σ_I^2) were calculated from the first 5 IPSCs of 20–40 IPSC trains (40Hz, ~30 repetitions). Plots of σ_I^2 versus $\bar{I}$ were fit with the equation describing the parabolic relation between σ_I^2 and $\bar{I}$ in the multinomial model of transmitter release:

$$\sigma_I^2 = \left[Q_p \bar{I} \frac{\bar{I}^2}{N} \right] (1 + CV_{QII}^2) + Q_p \bar{I} CV_{QI}^2$$

Where N is the number of release sites, Q_p is the mean quantal size, and CV_{QI} and CV_{QII} are the coefficients of variation of intrasite and intersite quantal variability, respectively (36). To estimate CV_{QI} , we used the variance of the last IPSC of the trains, which has strong synaptic depression and high failure rate, a scenario close to single release site transmission (36). We estimated CV_{QII} the total quantal variance CV_{QT} as:

$$CV_{QT} = \sqrt{CV_{QI}^2 + CV_{QII}^2}$$

and measuring CV_{QT} from stimulus-aligned IPSCs. The release probability (P) was calculated from:

$$P = \frac{\bar{I}}{Q_p N}$$

Extracellular stimulation

Focal stimulation was applied using theta-type capillary glass electrodes (open tip diameter ~3–5 μ m), filled with oxygenated ACSF, and connected via silver wires to a stimulation unit (World Precision Instruments). To elicit perisomatic IPSCs (Fig 5), stimulation was applied within 50 μ m of the recorded neuron soma in the presence of 10 μ M CNQX. The stimulus had 100 μ s duration and intensity adjusted (10–100 μ A) to reliably evoke a synaptic current when delivered at 0.2 Hz. Excitatory currents (Fig 1) were evoked in the presence of the GABA_AR antagonist gabazine (10 μ M).

Statistical analysis

Analysis was conducted using SPSS (IBM Analytics). Results are expressed as mean $\pm$ SEM. Shapiro-Wilk tests rejected normality of the distribution of the residuals for most samples. After natural logarithm transformation, Shapiro-Wilk tests produced $p > 0.05$ in all IPSC amplitude data samples. Thus, for the comparisons we used the log-transformed data in paired t-tests or mixed model repeated measures one-way ANOVA, with Sidak-adjusted post-hoc pairwise comparisons. In the Figures, the data are shown without log transformation. Because the IPSC failure rate was in many cases zero, which does not have a defined logarithm value, comparisons were performed using non-parametric Friedman's ANOVA.

Results

We recorded IPSCs evoked in connected pairs of presynaptic PV⁺ neurons and postsynaptic PCs located in mid/deep layer 3 of the PFC of G42 mice, which express GFP selectively in PV⁺ neurons (32, 37). All GFP-positive cells in PFC layer 3 had fast-spiking phenotype and were basket cells (Fig. 1A,B). No chandelier cells, which may be excitatory (38, 39), were found in layer 3. These findings are consistent with our recent study (34) that PV⁺ basket cells (PVBCs) are present in all cortical layers, but PV⁺ chandelier cells are present only in layer 2 (34). In all experiments, the NMDAR antagonist MK801 (1 mM) was applied to the postsynaptic PC via the pipette solution (40), to acutely block NMDARs solely in the recorded neuron (40). This approach enabled us to focus on NMDAR-mediated effects in the presynaptic PVBC, in the absence of any compensatory effects that may occur with long-term NMDAR alterations in NMDAR-deficient mice. We confirmed that intracellular MK801 blocked the postsynaptic NMDARs, since the NMDAR-mediated synaptic current recorded from PCs was abolished within ~8 min of intracellular MK801 application (Fig 1C,D).

To assess if NMDAR activation regulates the strength of PVBC-to-PC inhibition, we measured the effects of bath-applied NMDA (10 μ M) on the IPSCs in PVBC-to-PC pairs. We found that NMDA application increased the IPSC amplitude by ~40% (Fig. 2A–C), having maximal effect at ~4 min (Fig 2B). The effect was always reversible with NMDA washout (Fig. 2B–D). After 4 minutes of NMDA application, the IPSC was enhanced in 10 of 13 pairs (Fig 2C), and mixed model repeated measures One-Way ANOVA revealed a robust and significant reversible effect of NMDA on the IPSC amplitude ($F_{(2,10)}=25.31$ $p < 0.001$, Fig 2D). The NMDA effect was not correlated with age within the range examined here ($p=0.257$) nor with the control IPSC amplitude before NMDA application ($p=0.213$, Supplemental Fig S1).

To confirm that post-synaptic NMDARs were blocked during NMDA application, we measured changes in the holding current needed to voltage-clamp the postsynaptic PCs at -70 mV. Because NMDA elicits depolarizing currents in PFC PCs (41), NMDA application should increase the holding current if intracellular MK801 does not block the NMDARs. However, the holding current did not change during NMDA-mediated IPSC potentiation (before NMDA: -198.9 ± 92.4 pA, during NMDA -217.7 ± 106.4 , $n=13$, $p=0.413$). To also confirm that postsynaptic MK801 blocked the NMDARs, we measured the IPSC decay time,

since stimulation of NMDARs in the postsynaptic PC membrane near GABAergic synapses (42) should change the decay kinetics of GABA_AR-mediated currents (43). However, the exponential decay constant of the IPSCs did not change with NMDA (before NMDA: 7.43 ± 0.54 ms; during NMDA: 7.61 ± 0.41 , $n=13$, $p=0.608$).

If the NMDAR-mediated IPSC potentiation involves presynaptic mechanisms (i.e., higher release probability), then it may be associated with a lower rate of transmission failures. We found that, before NMDA application, the IPSCs at PVBC-to-PC inputs displayed substantial failure rates, as observed previously (33), and that NMDA reduced the failure rate reversibly (Fig. 3A,B). At PVBC synapses, manipulations that change the release probability change the failure rate, but do not significantly alter the paired-pulse ratio, contrasting with excitatory synapses (44). Similarly, we observed that the paired-pulse ratio was unaltered by NMDA (Supplemental Figure 2). We further assessed if the NMDA effect was consistent with a presynaptic mechanism of action, using multiple probability fluctuation analysis (MPFA) to measure the quantal parameters of transmission at PVBC-to-PC connections (36). Assuming a multinomial model of transmission, the quantal parameters were extracted fitting a parabolic function to plots of the experimental IPSC variance versus mean (Fig. 3C). We assessed the probability of release P , and the quantal size Q_p , before and after NMDA application, assuming that the number of release sites, N , remains constant. We found that NMDA did not change Q_p ($p=0.567$), but increased P ($p=0.047$) (Fig. 3D), in agreement with the increase in P suggested by the NMDA-mediated decrease in failures (Fig. 3A,B).

The changes in IPSC amplitude and failure rate induced by bath-applied NMDA despite concurrent postsynaptic NMDAR block, are consistent with an effect via NMDARs located in the presynaptic PVBC. However, the presynaptic effect may be indirectly mediated by NMDARs in other cell types (e.g. glia) (45). Thus, to determine if NMDARs located in the presynaptic PVBCs mediate the IPSC potentiation, we applied MK801 intracellularly for >10 minutes to both the postsynaptic PC and the presynaptic PVBC (Fig. 3E). In these conditions, neither the IPSC amplitude nor failure rate changed during NMDA application (Fig. 3E,F). Importantly, intracellular MK801 did not appear to produce NMDAR-independent non-specific effects because its application to the presynaptic PVBCs did not significantly alter most (8 of 11) of the PVBC membrane properties nor the IPSC properties (Supplemental Tables 1,2).

NMDAR activation might enhance transmitter release via a subthreshold depolarization of the dendrites that propagates to the axon terminals (29). Such NMDA-induced dendritic depolarization should be observable at the PVBC soma, *en route* to the axon. However, the membrane potential of the PVBC soma did not change during IPSC potentiation by NMDA application (control: -74.87 ± 1.86 mV; NMDA: -76.19 ± 1.86 mV; $n=13$, $p=0.1002$).

In previous work, the NMDAR antagonist AP5 decreased release at glutamate synapses on hippocampal (25) or neocortical (26, 27) PCs, suggesting that endogenous glutamate acting on presynaptic NMDARs enhances glutamate release. To determine if endogenous glutamate enhances PVBC-to-PC GABA release, we tested the effects of D,L-AP5 (100 μ M). D,L-AP5 did not significantly affect the amplitude or failure rate of IPSCs evoked in PVBC-to-PC

pairs (Fig. 4A,B). To further test if endogenous glutamate enhanced transmission at PV-to-BC synapses, we assessed the effects of intracellular MK801 application to the presynaptic PVBC on the IPSC failure rate. If endogenous glutamate potentiates GABA release, then MK801 in the presynaptic PVBC should increase the baseline failure rate. However, the IPSC failure rate did not differ between experiments with (0.067 ± 0.028) or without (0.036 ± 0.015 , $p=0.329$) MK801 in the presynaptic PVBC. These data suggest that baseline levels of endogenous glutamate at PVBC-to-PC synapses are not sufficient to enhance GABA release.

One possibility is that endogenous glutamate enhances release at GABA synapses only when the simultaneous activation of glutamate synapses elevates ambient glutamate levels. Extracellular stimulation was applied near the PC soma (Fig 5A), with AMPA-mediated transmission blocked by bath-applied CNQX (10 μ M), and MK801 applied intracellularly to the PCs clamped at -70 mV. Perisomatic stimulation may elicit IPSCs via perisomatic GABA synapses from either PV⁺ or cholecystokinin-positive (CCK⁺) interneurons (46). However, as in previous studies (47, 48), the IPSCs elicited by perisomatic extracellular stimulation (pIPSCs) were strongly inhibited by ω -agatoxin (Fig. 5A), which blocks GABA release from PV⁺ but not from CCK⁺ synapses (49–51). Moreover, both the ω -agatoxin-sensitive pIPSCs (Fig 5B) and pIPSCs evoked in the presence of ω -conotoxin (Fig 5C), which inhibits GABA release from CCK⁺ but not from PV⁺ synapses (49–51), were significantly enhanced by bath-applied NMDA. Finally, in contrast to the IPSCs in PVBC-to-PC pairs (Fig 4A,B), the pIPSCs were significantly depressed by AP5 application (Fig. 5D). Although we did not directly measure extracellular glutamate, the extracellular stimulation intensities used to elicit IPSCs typically elicit substantial glutamate release (Fig 1)(15). Moreover, as expected if ambient glutamate was elevated to levels sufficient to activate NMDARs at GABA synapses, AP5 significantly depressed the IPSCs evoked with extracellular stimulation (Fig 5D) but not in PVBV-to-PC pairs (Fig 4B). Our results are therefore consistent with the idea that when extracellular levels of endogenous glutamate are elevated above baseline, presynaptic NMDARs in perisomatic GABA synapses, likely from PVBCs, enhance inhibition of PCs.

Discussion

We report that NMDAR activation enhances the strength of synaptic inhibition of PCs by PVBCs in layer 3 of mouse PFC, via an effect apparently mediated by presynaptic changes at PVBC-to-PC synapses. This presynaptic NMDAR-mediated effect has important implications for the regulation of PFC network activity, and for our understanding of how NMDAR antagonists, or NMDAR hypofunction, affect this activity.

NMDAR activation in PVBCs enhances PVBC-to-PC synaptic inhibition via presynaptic mechanisms

Recent studies showing that presynaptic NMDARs regulate transmitter release at central synapses led us to hypothesize that NMDAR activation could enhance GABA release at PVBC-to-PC synapses, providing a potential mechanism for NMDAR antagonist-induced PC disinhibition (5). Studying synaptically-connected pairs in acute slices, we found that

NMDAR stimulation enhanced the strength of PVBC-to-PC synapses. Several lines of evidence suggest that this effect is mediated by presynaptic NMDAR-mediated regulation of GABA release. First, NMDA enhanced the IPSCs after postsynaptic NMDAR activation was prevented by intracellular application of MK801 (40). Second, the increase in IPSC amplitude with NMDAR activation was accompanied by a decrease in the rate of transmission failures, consistent with a higher probability of GABA release. Third, quantal analysis of transmission at PVBC-to-PC connections indicated that NMDAR activation increased the release probability without changing the quantal size. Fourth, the effects of NMDA application were blocked by MK801 application to the presynaptic PVBC. Thus, in concert with electron microscopy and electrophysiological studies, our results suggest that presynaptic NMDARs regulate GABA release at PFC synapses, as in the cerebellum and certain subcortical structures (28, 29).

The mechanisms by which NMDAR activation enhances the probability of GABA release remain unclear. Presynaptic effects of NMDA were suggested to be mediated by a subthreshold depolarization of the presynaptic neuron dendrites that propagates into the presynaptic boutons, enhancing transmitter release (29). Although a dendritic depolarization propagating into the axon should be measurable at the soma, we did not observe depolarization of the PVBC soma with NMDA application, suggesting that dendritic depolarization is not involved in the effects reported here. Alternatively, once NMDA or glutamate bind to NMDARs at a presynaptic locus, depolarization of the axonal bouton by the presynaptic action potential may relieve the voltage-dependent magnesium block, allowing calcium influx through the presynaptic NMDAR channels. Thus, NMDARs may enhance the intracellular calcium transient generated by voltage-gated calcium channels at the PVBC terminals, augmenting the probability of action potential-evoked GABA release.

Implications of the presynaptic NMDAR effect for the regulation of PFC network activity

A central finding of our study is that whereas NMDA significantly potentiates PVBC-to-PC GABA release, the competitive NMDAR antagonist AP5 does not affect PVBC-to-PC IPSCs, as also reported for somatosensory cortex (26). In contrast, AP5 application decreases glutamate release onto hippocampal or neocortical PCs (25, 26, 28, 29). Together, these findings suggest that baseline levels of glutamate are sufficient to regulate transmitter release via presynaptic NMDARs at glutamate synapses, but not at PVBC-to-PC GABA synapses. A previous study reported that NMDAR-mediated effects on IPSCs are age-dependent (52), but whether the IPSCs in such study originated from PVBCs is unknown. Because the NMDA effect on PVBC-to-PC synapses reported here was not age-dependent (Fig. S1), it is possible that the age-related NMDAR effects (52) involve other GABA synapses but not PVBC-to-PC synapses. Moreover, differences in age-dependence of presynaptic NMDAR effects may be partly explained by distinct NMDAR-mediated regulation of spontaneous versus evoked transmitter release (27). However we cannot rule out the possibility that, at development periods before the age range studied here, presynaptic NMDAR effects regulate IPSCs from PVBCs more strongly.

At glutamate synapses, presynaptic NMDARs might act as autoreceptors readily activated by synaptically-released glutamate (25, 26, 28, 29). In contrast, heterosynaptic NMDARs at

PVBC-to-PC GABA synapses are distant to glutamate release sites, and may be activated only when glutamate release is elevated above baseline levels, as suggested by our experiments testing the effects of AP5 on IPSCs evoked by perisomatic extracellular stimulation. We speculate, therefore, that the NMDAR-mediated potentiation of GABA release is a mechanism that adds strength to PVBC-mediated inhibition of PCs specifically when the levels of excitatory network activity are relatively high (Fig. 5E,F).

Our previous report that the NMDAR contribution to excitatory synaptic currents is smaller in PVBCs than PCs (15), suggests that NMDAR antagonism at excitatory synapses onto PVBCs does not mediate PC disinhibition. Can NMDAR antagonists disinhibit PCs via presynaptic effects at PVBC-to-PC synapses? The model shown in Fig 6A suggests that when network activity is below certain level, NMDAR antagonists may not affect GABA release because presynaptic NMDARs at PVBC-to-PC synapses are not exposed to glutamate. Low glutamate levels (Fig 6A) may be observed during gamma oscillations, when the PCs, as a population, are highly synchronized, but individual PCs are active only in some oscillation cycles, thus firing at low frequency (53–55). When excitatory network activity levels are high, presynaptic NMDARs at both PC-to-PC and PVBC-to-PC synapses may be exposed to glutamate (Fig 6B). This scenario may be observed in the PFC during the delay period of working memory tasks, when PCs sustain activity via recurrent excitation, and nearby PVBCs provide inhibitory gain control (56). Presynaptic NMDARs at PC-to-PC synapses may facilitate recurrent excitation, and those at PVBC-to-PC synapses may enhance gain control. However, due to the essential role of postsynaptic NMDARs in delay period-related recurrent excitation (57, 58), it may be expected that general NMDAR antagonism would decrease the excitatory drive onto PCs, thus reducing delay period activity. Consistent with this idea, instead of producing disinhibition, systemic or intra-PFC NMDAR antagonist administration significantly reduced delay-related firing in monkey PFC during a working memory task (59). In mice, PFC neurons display working memory task-related activity (56, 60), and working memory is impaired in rats by systemic or intra-PFC NMDAR antagonist administration (61, 62). Whether NMDAR antagonists affect working memory task-related activity in rat or mouse PFC remains untested. Moreover, whether different PC/PVBC sub-networks are engaged in gamma oscillations or persistent activity during the delay period is unknown. The validity of the model in Fig 6, however, depends on characterizing the physiological conditions that elevate the endogenous glutamate levels to activate presynaptic NMDARs at PVBC-to-PC synapses.

PVBC-to-PC inhibition may be recruited by the local circuit, as discussed above, or by thalamic, hippocampal or amygdala inputs, which monosynaptically target inhibitory interneurons, including PV⁺ cells (21, 63–66). These inputs may be crucial for cognitive processes (67, 68), by eliciting feed-forward inhibition to shape the timing of PC spiking (69). Importantly, thalamic and amygdala inputs elicit weak postsynaptic NMDAR currents in PVBCs (13, 19, 21), suggesting that feed-forward inhibition via PVBCs is primarily AMPA receptor-mediated. Thus, while NMDAR activation patterns in vivo and in vitro may differ significantly, our current results imply a major role of presynaptic NMDARs at PVBC-to-PC GABA synapses. Together with our previous findings (15), and data from others (11–22), the data reported here suggest that PC disinhibition by NMDAR antagonists in awake rats (5) is not mediated by PVBCs.

Could PC disinhibition by NMDAR antagonists (5) be mediated by inhibitory neuron subtypes different from PVBCs? We suggested previously (15) that NMDAR antagonist-induced disinhibition may be in part mediated by low-threshold spiking (LTS) interneurons, which display large postsynaptic NMDA currents (12, 14). LTS interneurons include somatostatin-positive (SST⁺) cells which are also abundant in neocortex (70), and similarly play a crucial role in PC inhibition (71). Thus, NMDAR antagonists may disinhibit PCs by reducing SST⁺ neuron activity. SST⁺ cells directly inhibit PV⁺ neurons (72), and may be involved in gamma band oscillations (73). NMDAR antagonists may therefore decrease SST⁺ cell activity, disinhibiting both PCs and PV⁺ neurons (74), and disrupting gamma oscillations (73). SST⁺ cells are strongly inhibited by vasointestinal peptide-positive (VIP⁺) interneurons, but whether NMDARs are important to recruit VIP⁺ interneurons is unknown. Thus, much needs to be learned on how NMDARs regulate the activity flow in PFC microcircuits, before a circuit-based model that explains PC disinhibition by NMDAR antagonists in awake rats (5), or by NMDAR hypofunction in schizophrenia, can be developed.

Independent of PC disinhibition, our data suggest that NMDAR hypofunction at PVBC-to-PC synapses in schizophrenia might disrupt a crucial mechanism that normally enhances PC inhibition when the PFC network is engaged in working memory-related operations. However, empirical support for NMDAR hypofunction in PV⁺ neurons in the cortex of subjects with schizophrenia is scarce, and limited to two publications showing reduced levels of GluN2A subunit mRNA at the PV⁺ neuron soma (75, 76). PVBC-to-PC synapses have not been studied in mice with NMDA-deficient PV⁺ neurons. Such studies would be highly informative, although those mouse models produced some conflicting results (77–81). To assess the relevance of our current findings for the pathophysiology of schizophrenia it would be important to characterize the NMDAR subunits present presynaptically in PVBC-to-PC synapses, to determine if their levels are altered in the PFC of individuals with schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Intrinsic physiology and morphology of the PV⁺ interneurons recorded in PFC layer 3, and test of postsynaptic NMDAR block by intracellular MK801 application. **(A)** Response of a representative layer 3 PV⁺ neuron to a series of hyperpolarizing and depolarizing rectangular current steps, illustrating the fast-spiking phenotype. **(B)** Morphological reconstructions of the layer 3 PV⁺ interneurons, showing properties consistent with those of basket cells. Soma and dendrites are shown in red and the axon in blue. **(C)** EPSCs evoked by extracellular stimulation in PCs in the presence of the GABA_A receptor antagonist gabazine (10 μ M) and recorded at different holding potentials, in control conditions (left panel), or after at least 10

minutes of whole-cell recording in the presence of 1 mM MK801 in the pipette solution (right panel). In the control experiment (left panel), the NMDAR component of the EPSC is revealed by the marked prolongation of the EPSC decay time when holding the PC at +60 mV (gray trace) versus -80 mV (black trace). With MK801 in the pipette solution (right panel), the EPSC prolongation at +60 mV is abolished (green trace), and addition of the NMDA antagonist D,L-AP5 (100 μ M) does not produce further change in the EPSC (blue trace). For ease of comparison, all traces were scaled to the same peak amplitude. **(D)** Time course of the effect of intracellular MK801 on the NMDA component of evoked EPSCs by extracellular stimulation. At time=0, the PCs were voltage clamped at +60 mV, and EPSCs were evoked for 8 min at 0.2 Hz. The plots on the left show scaled representative traces of one recording showing the acceleration of the EPSC decay by NMDAR block over the course of the experiment. The absence of NMDA current in the EPSC is demonstrated by the lack of effect of bath-applied D,L-AP5 (100 μ M) after a 10 min application (blue trace). The plot on the right shows, for EPSCs recorded holding the cell at +60 mV (means $\pm$ SEM, n=7), the time course of the amplitude of the EPSC measured at 80 ms from the stimulation, and normalized relative to the EPSC peak amplitude.

Figure 2.

NMDAR activation enhances the strength of inhibitory transmission in PVBC-to-PC connected pairs. **(A)** Top left: Infrared-Differential Interference Contrast image illustrating a recorded PVBC-to-PC pair. Bottom left: schematic drawing of the recording configuration. Right: an action potential (black trace) elicited in a PVBC by a depolarizing current step (not shown), and corresponding averages of IPSCs evoked in the PC before (control, green), during application of 10 μ M NMDA (NMDA, blue), and after washout (wash, green). **(B)** Time course plots illustrating the effect of bath-applied NMDA on the IPSC amplitude. The gray lines show data from individual experiments, and the data points show the means $\pm$ SEM ($n=8-13$). The data in each experiment were normalized to the baseline average. For most experiments, the effect of NMDA peaked at 4 minutes of application. **(C)** Summary plot of IPSC amplitude normalized relative to baseline control value (control), at 4 min of NMDA application (NMDA), and during washout of NMDA (wash). The wash values were obtained by averaging the last 4 IPSCs recorded during washout. Each data point represents the average IPSC for each condition in each experiment. The data from the same PVBC-to-PC pair are joined by lines, and the black bars indicate the mean values. Note that at 4 minutes of NMDA application the IPSC amplitude was enhanced relative to baseline for 10

of 13 of the PVBC-to-PC pairs. **(D)** Summary plot showing the significant and reversible increase (4 minutes of NMDA application), in the amplitude of the IPSC evoked in PCs by presynaptic PVBCs (Mixed Model One-Way ANOVA, $F_{(2,10)}=25.315$, $p<0.001$, $n=8-13$). Each data point represents the average IPSC for each condition in each experiment. The data from the same PVBC-to-PC pair are joined by lines. The differences between groups were assessed by Sidak-adjusted post hoc pairwise comparisons.

Figure 3.

NMDARs located in the presynaptic PVBC are required for the potentiation of IPSCs by NMDA application. **(A)** NMDAR activation decreased the failure rate of IPSCs in PVBC-to-PC connections. IPSCs in a PVBC-to-PC pair, illustrating individual responses (gray traces) and their averages, recorded before (control, green), during 10 μ M NMDA application (NMDA, blue) and after NMDA washout (wash, green). Transmission failures, represented by gray traces without response, are observed in control and wash conditions, but not under NMDA. **(B)** Summary plot showing the significant and reversible decrease in the IPSC failure rate by NMDA application (Friedman's ANOVA, Chi-Square=7.913 $p=0.019$, $n=10$).

Each data point represents the average IPSC for each condition, in each experiment, data from the same PVBC-to-PC pair are joined by lines. Indicated above the plot are the p values from the post hoc pairwise comparisons. (C) Representative plots illustrating the fitting of parabolic functions (black lines) to plots of IPSC mean and variance in a PVBC-to-PC pair before (control, green) and during NMDA application (NMDA, blue). These plots are employed to extract the quantal parameters of PVBC-to-PC transmission estimated by multiple probability fluctuation analysis (MPFA). (D) Bar graphs summarizing the effects of NMDA application on the probability of release (P, left panel), and quantal size (Q_p , right panel), estimated by MPFA in control and NMDA conditions. Paired t-test comparisons revealed that NMDA application caused a significant increase in P ($p=0.046$), without changes in Q_p ($p=0.567$). Data are means $\pm$ SEM ($n=13$). (E) The effect of NMDA is abolished by application of MK801 to the presynaptic PVBCs. Top, schematic drawing of the recording configuration, with MK801 included in the pipette solution used to record both the presynaptic PVBC and the postsynaptic PC. Bottom, representative traces showing action potentials evoked by current steps in a presynaptic PVBC and the IPSCs evoked in the postsynaptic PC (thin gray traces, individual IPSCs; thick traces, average IPSC) before (control, green) and during bath application of 10 μ M NMDA (NMDA, blue). (F) The IPSC amplitude (top panel) and failure rate (bottom panel) were not altered by NMDA application with MK801 present in the pipette solution used to record both neurons in the pair. The significance of the difference between control, NMDA and wash conditions was tested using mixed model repeated measures ANOVA ($F_{(2,8)}=0.02$, $p=0.980$, $n=8-11$) for the IPSC amplitude, and for the IPSC failure rate (Friedman's ANOVA, Chi-Square=0.545, $p=0.761$). Each data point represents the average IPSC for each condition in each experiment, and continuous lines join data from the same PVBC-to-PC pair.

Figure 4.

The NMDAR antagonist D,L-AP5 does not affect the IPSCs in PVBC-to-PC pairs. (A) Top, schematic drawing of the recording configuration. Bottom, action potentials evoked by current steps in a preynaptic PVBC, and the IPSCs evoked in the postsynaptic PC (thin traces, individual IPSCs; thick traces, average IPSC) in control (green) and with 100 μM D,L-AP5 (blue). (B) The IPSC amplitude and failure rate were not significantly affected by D,L-AP5 application. Top, summary plot of IPSC amplitude for each PVBC-to-PC pair in control, AP5 and wash, revealing a lack of significant change in the IPSC amplitude ($F_{(2,7)}=2.431$, $p=0.118$, $n=7-10$, mixed model repeated measures ANOVA). Each data point represents the average IPSC for each condition in each experiment. Data from the same PVBC-to-PC pair are joined by lines. Center, time course plot of IPSC amplitude relative to baseline average during control, AP5 and washout. Data are means $\pm$ SEM ($n=7-10$). Bottom, IPSC failure rate measured for each pair in control, NMDA and wash conditions

(Friedman's ANOVA, Chi-Square=1.368 $p=0.504$). Each data point represents the average IPSC for each condition in each experiment, data from the same PVBC-to-PC pair are joined by lines.

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Figure 5.

The IPSCs evoked in PCs by perisomatic extracellular stimulation are modulated by both NMDA and AP5. **(A)** IPSCs elicited by perisomatic focal extracellular stimulation (pIPSCs) in the presence of the AMPAR antagonist CNQX (10 μ M), are almost completely blocked by ω -agatoxin, which inhibits GABA release from PV⁺ neurons. Left, schematic drawing of the experimental design; ext. stim. : perisomatic extracellular stimulation. Center, representative pIPSCs evoked in control before application and in the presence of ω -agatoxin (thin lines, individual responses; thick lines, averages). Right, time course plot of pIPSC amplitude before and during ω -agatoxin application. **(B)** Top, plots of pIPSC

amplitude relative to the baseline average (control), measured after 4 minutes of NMDA application (NMDA), and during NMDA washout (wash). Each symbol represents the average IPSC for each condition, continuous lines join data from the same experiment. The black bars indicate the mean values. Note that the pIPSCs were reversibly enhanced by NMDA. Bottom, plots of absolute pIPSC for the same experiments as above (mixed model repeated measures ANOVA, $F_{(2,10)}=4.007$, $p=0.031$, $n=12-13$). (C) NMDA enhanced the pIPSCs evoked in the presence of ω -conotoxin (ω -ctx). Shown are plots of relative (top) and absolute (bottom) pIPSC amplitude as in (B). ($F_{(2,15)}=6.760$, $p=0.003$, $n=15-18$, mixed model repeated measures ANOVA). (D) Bath-applied D,L-AP5 (100 μ M) reduced the amplitude of the pIPSC ($F_{(2,15)}=7.201$, $p=0.003$, $n=15-18$, mixed model repeated measures ANOVA). Shown are plots of relative (top) and absolute (bottom) pIPSC amplitude as in (B) and (C).

Figure 6.

A model to interpret the functional relevance of the regulation of PVBC-to-PC inhibitory strength by presynaptic NMDARs. (A) When local excitatory activity and glutamate release are low, as during paired recordings, ambient glutamate levels at PVBC-to-PC GABA synapses are not sufficient for presynaptic NMDAR activation. (F) When local excitatory activity and glutamate release increase above a threshold level (a situation produced by local extracellular stimulation in our experiments), ambient glutamate levels are elevated, and reach the presynaptic PVBC terminal, producing NMDAR activation and potentiation of GABA release.