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Prolonged exposure to NMDAR antagonist induces cell-type specific changes of glutamatergic receptors in rat prefrontal cortex

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ABSTRACT

N-methyl-D-aspartic acid (NMDA) receptors are critical for both normal brain functions and the pathogenesis of schizophrenia. We investigated the functional changes of glutamatergic receptors in the pyramidal cells and fast-spiking (FS) interneurons in the adolescent rat prefrontal cortex in MK-801 model of schizophrenia. We found that although both pyramidal cells and FS interneurons were affected by in vivo subchronic blockade of NMDA receptors, MK-801 induced distinct changes in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors in the FS interneurons compared with pyramidal cells. Specifically, the amplitude, but not the frequency, of AMPA-mediated miniature excitatory postsynaptic currents (mEPSCs) in FS interneurons was significantly decreased whereas both the frequency and amplitude in pyramidal neurons were increased. In addition, MK-801-induced new presynaptic NMDA receptors were detected in the glutamatergic terminals targeting pyramidal neurons but not FS interneurons. MK-801 also induced distinct alterations in FS interneurons but not in pyramidal neurons, including significantly decreased rectification index and increased calcium permeability. These data suggest a distinct cell-type specific and homeostatic synaptic scaling and redistribution of AMPA and NMDA receptors in response to the subchronic blockade of NMDA receptors and thus provide a direct mechanistic explanation for the NMDA hypofunction hypothesis that have long been proposed for the schizophrenia pathophysiology.

1. Introduction

Glutamatergic hypotheses of schizophrenia are based on the ability of *N*-methyl-D-aspartic acid (NMDA) receptor antagonists such as phencyclidine, ketamine, or MK-801 to induce psychotic symptoms closely resembling those of schizophrenia (Farber, 2003; Javitt, 2004; Javitt and Zukin, 1991; Jentsch and Roth, 1999; Krystal et al., 1994; Lahti et al., 1995; Marino and Conn, 2002; Moghaddam, 2003; Olney and Farber, 1995; Pinault, 2008). Although the exact mechanisms remain elusive, it is generally believed that NMDA antagonists reduce the stimulation of NMDA receptors in γ-aminobutyric acid-ergic (GABAergic) interneurons, leading to disinhibition of glutamatergic pyramidal neurons, which in turn induces hyper-excitability in pyramidal neurons and thus excessive glutamate release in the limbic systems (Benes, 2000; Jentsch and Roth, 1999; Lisman et al., 2008; Moghaddam et al., 1997; Olney et al., 1991). The critical players in this hypothesis are the GABAergic interneurons, which play an essential role in the regulation of activities of pyramidal cells in the cortical circuits (Lisman et al., 2008; Nakazawa et al., 2011). Indeed, clear deficits of GABAergic transmissions were reported in the brains of patients with schizophrenia (Lewis et al., 2005). Deficient GABA function is known to be closely associated abnormal oscillations and synchrony that are critical for the generation of cognitive deficits and other symptoms of the disorder (Uhlhaas and Singer, 2006, 2010). In animal models, repeated application of NMDA receptor antagonists evokes behavioral and neurochemical changes (Carpenter and Koenig, 2008; Gunduz-Bruce, 2009; Lindsley et al., 2006; Rujescu et al., 2006). For example, subchronic administration of NMDA receptor antagonists decreases the expression of parvalbumin (PV) in GABAergic interneurons (Abekawa et al., 2007; Braun et al., 2007; Cochran et al., 2003; Coleman et al., 2009), disrupts cortical inhibition (Grunze et al., 1996; Li et al., 2002; Zhang et al., 2008), induces working memory deficits (Coleman et al., 2009), attentional deficits and increased impulsivity (Amitai and Markou, 2009; Amitai et al., 2007).

Abbreviations: CI-AMPARs, calcium-impermeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; CP-AMPARs, calcium-permeable AMPA receptors; D-AP5, D-(−)-2-amino-5-phosphonopentanoic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EPSCs, excitatory postsynaptic currents; FS, fast-spiking; GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartic acid; PFC, prefrontal cortex; RI, rectification index.

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Our recent studies showed that excitatory inputs onto immature fast-spiking (FS) interneurons have strong NMDA receptor-mediated currents that progressively weaken with age, becoming small or absent in adult FS interneurons in the rat prefrontal cortex (PFC) (Wang and Gao, 2009, 2010). These properties suggest that FS interneurons may be particularly vulnerable to environmental or drug stimulation (e.g., MK-801) during cortical development (Xi et al., 2009b). Indeed, in a strain of genetically modified mice in which the NMDAR subunit NR1 was conditionally knocked out in GABA neurons, including PV-positive cells, failed to produce significant effects when the knockout occurred after adolescence. However, when the deletion was conducted early in development, adult mice developed schizophrenia-like behavioral alterations (Belforte et al., 2010). Despite this large body of evidence, it remains unclear how NMDA receptor antagonist affects the synaptic functions of prefrontal neurons, particularly in FS interneurons. It is also unknown why GABAergic interneurons are more vulnerable to NMDA receptor antagonist among different cell-types (Wang et al., 2008a) and how the proposed hypofunction of glutamatergic systems occur in the pathogenesis of schizophrenia, particularly during the adolescent period. We therefore explored how sub-chronic treatment with MK-801 resulted in the disruption of glutamatergic transmission in adolescent rat prefrontal neurons by comparing the changes of NMDA and AMPA receptors in FS interneurons with those in pyramidal neurons. We found that MK-801 induced distinct cell-type specific alterations of glutamatergic receptors in the pyramidal neurons and FS interneurons.

2. Materials and methods

2.1. Animal treatment

We used 98 female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) at adolescent ages PD30 to PD39 (Spear, 2000; Tseng and O'Donnell, 2007; Wang and Gao, 2010). The rats were maintained on a 12-h light/dark cycle and were fed *ad libitum*. To be consistent, we continued to use female rats in which we have reported significant changes of NMDA receptors in the MK-801 model (Xi et al., 2009a, 2011, 2009b). In fact, although we did not observe a significant gender difference, some studies suggested that PCP-, MK-801-, and ketamine-induced effects are more reproducible in female animals (Dickerson and Sharp, 2006; Farber et al., 1995; Nakki et al., 1996). The animals were treated under National Institutes of Health animal use guidelines, and the experimental protocol was approved by the Institutional Animal Care and Use Committee at Drexel University College of Medicine. Because we could use only one rat each day for electrophysiological recording, the rats were treated with either MK-801 (0.1 mg/kg, intraperitoneally [i.p.], daily) or 0.9% saline as vehicle control for 5 consecutive days from PD30 to PD39. This dosage is based on our recent reports in which 0.1 mg/kg MK-801 were found to be effective in inducing changes of NMDA receptors in both transcription and protein levels (Xi et al., 2011, 2009b).

2.2. Cortical slice preparation

The detailed procedure is described in our previous publications (Gao et al., 2001; Wang and Gao, 2009, 2010). The rats (at ages of PD36-45) were deeply anesthetized with Euthasol (0.2 ml/kg, *i.p.*) 24 h after the last injection of MK-801 or saline. The rats were rapidly perfused with ice-cold (<4 °C) sucrose solution filled with 95% O_2 and 5% CO_2 and then were decapitated. The sucrose solution contained (in mM) NaCl 87; KCl 2.5; NaH_2PO_4 1.25; $NaHCO_3$ 25; $CaCl_2$ 0.5; $MgSO_4$ 7.0; sucrose 75; and glucose 25. The brains were quickly removed and immersed in ice-cold sucrose solution. The frontal cortex was cut into 300-μm slices with a Leica Vibratome (VT 1000S; Leica, Bannockburn, IL), and the brain slices were incubated in oxygenated sucrose solution at 35 °C for 1 h. The cortical slices were kept at room temperature until being transferred into a submerged chamber for recording. The recording chamber was perfused with Ringer's solution bubbled with 95% O_2 and 5% CO_2 at a perfusion rate of 2–3 ml/min. The Ringer's solution contained (in mM) NaCl 128; KCl 2.5; NaH_2PO_4 1.25; $CaCl_2$ 2; $MgSO_4$ 1.0; $NaHCO_3$ 26; and dextrose 10.

2.3. Electrophysiological recordings

Whole-cell patch-clamp recordings were conducted in the prelimbic region through an upright microscope (Olympus BX51WI, Olympus Optics, Japan) equipped with infrared-differential interference contrast optics. The recordings were conducted at ~35 °C, and the resistance of the recording pipette (1.2 mm borosilicate glass, Warner Instruments Inc., Hamden, CT) was 4.5–7 MΩ. As we recently reported (Wang and Gao, 2009, 2010), the pipette tips were first filled with a K^+-gluconate-based intracellular solution and then back-filled with Cs^+-containing solution. The K^+-gluconate solution contained (in mM) K^+-gluconate 120; KCl 6; ATP-Mg 4; Na_2GTP 0.3; EGTA 0.1; Hepes 10; and 0.3% biocytin (pH 7.3), whereas the Cs^+-solution contained (in mM) Cs-gluconate 120; lidocaine 5 (QX-314); $CsCl_2$ 6; ATP-Mg 1; Na_2GTP 0.2; Hepes 10; and 0.3% biocytin (pH 7.3, adjusted with CsOH). With this method, we were able to successfully record the action potentials (AP) immediately after forming a giga-seal. The membrane potentials were corrected for liquid junction potential (9.8 mV) of the K^+-gluconate solution.

To record the miniature excitatory postsynaptic currents (mEPSCs), we stabilized the neurons in regular Ringer's solution for 5 min to allow the Cs^+ diffusion into the neurons, and the spontaneous synaptic currents were continuously recorded for 5 min at a holding potential of −70 mV in the presence of picrotoxin (PTX; 50 μM, Sigma-Aldrich, St. Louis, MO) to block GABAergic transmissions. The mEPSCs were then recorded with the addition of tetrodotoxin (TTX, 1 μM, Ascent Scientific, Princeton, NJ) to block spontaneous action potential-induced synaptic currents. Twenty rats were used for this study. In one set of experiment ($n = 8$ rats), we recorded mEPSCs in pyramidal cells with 1 mM MK-801 loaded into recording pipette to block postsynaptic NMDA receptors; whereas in another group of rats ($n = 10$ rats), we examined mEPSC changes and spike properties following a single MK-801 (0.1 mg/kg, i.p.) treatment. In some neurons, selective NR2B antagonist ifenprodil (3 μM) were bath-applied to examine the effects on mEPSC amplitude and frequency, which would reflect the subunit components of presynaptic NMDA receptors.

The evoked EPSCs were recorded in layer II/III neurons of the PFC by stimulating layer II/III with a bipolar electrode placed ~300 μm away from the recorded cells. To examine presynaptic NMDA receptors, we first applied paired-pulse (0.1 ms, 10–100 μA, 0.1 Hz, inter-stimulus interval 50 ms) to evaluate paired-pulse ratio (PPR) changes before (pre-AP5) and after AP5 treatment ($n = 9$ rats). In addition, we also we induced EPSCs with minimal stimulation which produced about 50% failures in all stimulus trials to observe the changes of percent failures after AP5 treatment ($n = 6$ rats).

To examine the AMPA receptor subunit changes in MK-801 model, we calculated rectification index (RI) as recently reported (Wang and Gao, 2010). A single pulse (0.1 ms, 10–100 μA, 0.1 Hz) was applied for the extracellular stimulation. The current–voltage (I–V) curve of the AMPA receptor-mediated EPSCs was determined by recording at −60, 0, and +60 mV in Ringer's solution containing PTX and the NMDA receptor antagonist AP5 (50 μM, Tocris Bioscience, Ellisville, MO) ($n = 32$ rats).

In addition, in one set of experiment ($n = 6$ rats), the recorded neurons were clamped at −70 mV and bathed with PTX and AP5 to isolate AMPA receptor-mediated current. Then calcium-permeable AMPA receptor (CP-AMPAR) antagonist 1-naphthylacetyl spermine trihydrochloride (NASPM, 50 μM) was bath-applied to examine the changes in AMPA receptor-mediated currents in MK-801 model.

To investigate the Ca^{2+} permeability of AMPA receptor-mediated currents in MK-801 model, the normal Ringer's solution containing 2 mM Ca^{2+} was replaced with a solution containing 30 mM Ca^{2+}. The control (2 mM Ca^{2+}) and highly concentrated Ca^{2+} (30 mM) solutions were identical in composition except for the concentrations of $CaCl_2$ and NaCl, which (in mM) were 2 and 124 and 30 and 92.7, respectively. The evoked AMPA-EPSCs were first recorded in the control solution and then in the high-concentration Ca^{2+} solution for 10 min in the presence of PTX (50 μM) ($n = 8$ rats).

All electric signals were recorded with a MultiClamp 700B (Molecular Devices, Union City, CA) and acquired at sampling intervals of 20–50 μs through a DigiData 1322A and pCLAMP 9.2 software (Molecular Devices). The access resistances were constantly monitored through a pulse of negative 5 mV (200 ms duration) and were adjusted during recordings as needed.

2.4. Data analysis

The passive membrane properties in pyramidal cells and FS interneurons were measured as reported in our previous studies (Wang and Gao, 2009, 2010). These parameters included resting membrane potential, input resistance, membrane time constant, AP threshold, AP half-width, and afterhyperpolarization (AHP). The fast AHP and late AHP were analyzed on the basis of a previous report (Storm, 1987). The value of fast AHP was determined as the difference between the AP threshold and the lowest point of the first undershoot of the spike, whereas the late AHP was the second deep valley after the spike.

The mEPSCs recorded in the voltage-clamp mode were analyzed with Clampfit 9.2. A typical mEPSC was selected to create a sample template for event detection within a data period, and the AMPA mEPSCs were detected with a threshold set at three times the value of the root mean square of the baseline noise. The event numbers were sorted to make histogram at bin size of 2 pA and the data were fitted with Gaussian function in Clampfit 9.2. The rectification index (RI) of the evoked AMPA-EPSCs was calculated as the amplitude of AMPA-$EPSC_{+60\ mV}$/AMPA-$EPSC_{-60\ mV}$ in the extracellular stimulus recording.

2.5. Statistical analysis

For all experiments, treatment effects were statistically analyzed by one-way ANOVA, followed by the appropriate *post hoc* tests using the software SPSS 16.0.

Paired Student's *t* test was used when comparisons were restricted to two means in the neuronal samples (e.g., pre-AP5 and AP5), whereas unpaired nonparametric tests (Mann–Whitney) were used to compare the medians of two groups of non-parameter samples (i.e., the spike numbers). The Kolmogorov–Smirnov (K–S) analysis was applied to analyze the amplitude and interevent interval of mEPSCs. Error probability of $p < 0.05$ was considered to be statistically significant, and the data were presented as mean ± standard error.

3. Results

3.1. Subchronic MK-801 induces similar changes in membrane properties in pyramidal cells and FS interneurons

Previous studies have indicated that treatment with a single dose of MK-801 induces significant changes in the firing properties of APs in the prefrontal cortical neurons in freely moving animals (Homayoun and Moghaddam, 2007; Jackson et al., 2004) and acute MK-801 produces opposite effects on pyramidal neurons and GABAergic interneurons (Homayoun and Moghaddam, 2007). In addition, we recently reported that pyramidal cells and FS interneurons in the PFC exhibit different subtypes of NMDA receptors (Wang and Gao, 2009; Wang et al., 2008b; Xi et al., 2009a). Notably, individuals that use NMDAR antagonist repeatedly, rather than acutely, display hypofrontality in functional imaging studies (Jentsch and Roth, 1999; Jentsch et al., 1997c; Pratt et al., 2008). Furthermore, the symptoms related to schizophrenia are more severe and enduring than after acute treatment with PCP in rats (Gilmour et al., 2011; Pratt et al., 2008). This suggests a dissociation of the acute and chronic effects of NMDA receptor antagonism on brain mechanisms particularly in relation to PFC activity and cognitive deficits (Gilmour et al., 2011; Pratt et al., 2008). We therefore wondered whether subchronic treatment with MK-801 would cause differential changes of membrane properties in the prefrontal neurons. All recordings were carried out in layer II/III of the medial PFC, and the morphological characteristics of all recorded cells were initially identified under infrared-differential interference contrast optics visualization. Pyramidal neurons showed cone-shaped soma and typical apical dendrites toward cortical layer I, whereas FS interneurons had round, oval, or elongated somas. These two kinds of neurons can also be distinguished by their distinctly different firing patterns that were immediately (<1 min) recorded with step currents after the giga-seal formation in the patch-clamp recordings (Fig. 1A). The pyramidal neurons in both the vehicle control and the MK-801 model responded to the injection of the depolarizing somatic current with regular and frequency-adapting action potentials whereas FS interneurons were characterized by high-frequency fast firing without adaptation. No spontaneous firings were observed at the resting conditions in either the FS interneurons or the pyramidal neurons in the control, but spontaneous firing occurred in the MK-801 model in both cell types under resting conditions. The spike (AP) numbers of pyramidal neurons and FS interneurons in the MK-801-treated rats were not significantly different overall compared with those from the saline vehicle controls (two-way ANOVA: $n = 7$; $F = 0.975$, $p = 0.448$ for pyramidal cells; $n = 5$, $F = 1.022$, $p = 0.427$; Fig. 1B). The input resistances were significantly increased ($p < 0.05$) in pyramidal cells but not in FS interneurons ($p > 0.05$) compared with those in vehicle control cells. As shown in Table 1 and Fig. 1C, the resting membrane potentials of both pyramidal neurons and FS interneurons were significantly depolarized in the MK-801 model ($p < 0.005$ for both pyramidal cells and FS interneurons). Two-way ANOVA analysis indicated that the membrane potential changes in MK-801 model were significantly different in both pyramidal cells and FS interneurons at current steps from −300 pA to +50 pA compared with those in saline controls (P: $n = 7$ for both control and MK-801, $F = 4.764$, $p < 0.001$; FS: $n = 5$ for both control and MK-801, $F = 2.943$, $p < 0.01$; Fig. 1C). The membrane time constant and AP half-width of pyramidal neurons in MK-801 model exhibited no significant changes compared with those in the controls. In contrast, the membrane time constant of FS interneurons decreased ($p < 0.005$) whereas the AP half-width of FS cells in the MK-801 model significantly increased compared with those in the controls ($p = 0.001$). Nevertheless, the patch-clamp recordings in both pyramidal cells and FS interneurons in the MK-801 model could last at least 30 min in most of the neurons recorded although the membrane properties were changed by treatment with MK-801.

3.2. MK-801 induces distinct changes of AMPA receptor-mediated miniature postsynaptic currents in pyramidal cells and FS interneurons

To further explore the effects of MK-801 on the synaptic transmission, AMPA receptor-mediated mEPSCs were recorded from the pyramidal cells and FS interneurons under conditions of saline vehicle control and MK-801 treatment. The AMPA mEPSCs were pharmacologically isolated by bath application of the $GABA_A$ receptor antagonist PTX (50 μM) and the sodium channel blocker TTX (1 μM) at a holding potential of −70 mV. The mEPSCs were detected as fast inward currents (Fig. 2A) and could be completely blocked by the AMPA receptor antagonist DNQX (20 μM, data not shown). The basic properties of the AMPA mEPSCs in both FS interneurons and pyramidal neurons, including the rise time ($p > 0.05$ for both pyramidal cells and FS interneurons) and the decay kinetics (see scaled overlaps in Fig. 2B, $p > 0.05$ for both pyramidal cells and FS interneurons), exhibited no significant differences. However, both mEPSC amplitude and frequency recorded from the pyramidal cells were significantly increased in the MK-801 model compared with those in the controls ($n = 9$ for both control and MK-801, $p < 0.05$ for amplitude and $p < 0.01$ for frequency; Fig. 2B–D left panels).In contrast, in the FS interneurons, the mEPSCs amplitude was significantly decreased by about 50% (19.1 ± 2.10 pA in control vs. 9.30 ± 0.85 pA in MK-801, $n = 7$ for both, $p < 0.001$; Fig. 2C right panel), whereas the interevent intervals (frequency) of the mEPSCs were not significantly changed although there was a tendency of decrease in MK-801 model ($p > 0.05$; Fig. 2D right panel) compared with those in the vehicle control. It should be noted that the frequency change could also derive from changes in postsynaptic AMPA receptors. Because MK-801 induced significant changes of membrane properties in FS interneurons, it is possible that the synaptic efficacy was altered in FS interneurons, as shown in Fig. 2B (right panel). The decreased amplitude of the mEPSCs suggested a possible postsynaptic hypofunction of AMPA receptors, whereas the unchanged frequency of mEPSCs might be attributable to an unaltered presynaptic glutamate release. These results suggest that FS interneurons were indeed in the state of hypofunction, whereas pyramidal neurons were altered with an increased release of glutamate and postsynaptic AMPA receptor-mediated activity in the MK-801 model, as previously proposed (Javitt, 2004; Lisman et al., 2008; Millan, 2005; Moghaddam and Jackson, 2003; Olney and Farber, 1995).

To examine whether there are similar changes after a single MK-801 treatment, we recorded mEPSCs in both pyramidal neurons and FS interneurons 24 h after a single injection of MK-801 ($n = 9$ rats at PD30-39) compared with those in 5-day injection. As shown in Supplemental Fig. 1, we found that, interestingly, there are no significant differences in both mEPSC amplitude and frequency in either pyramidal neurons or FS interneurons. This result suggests that even single injection of MK-801 could induce clear alteration in AMPA receptors in the prefrontal neurons.

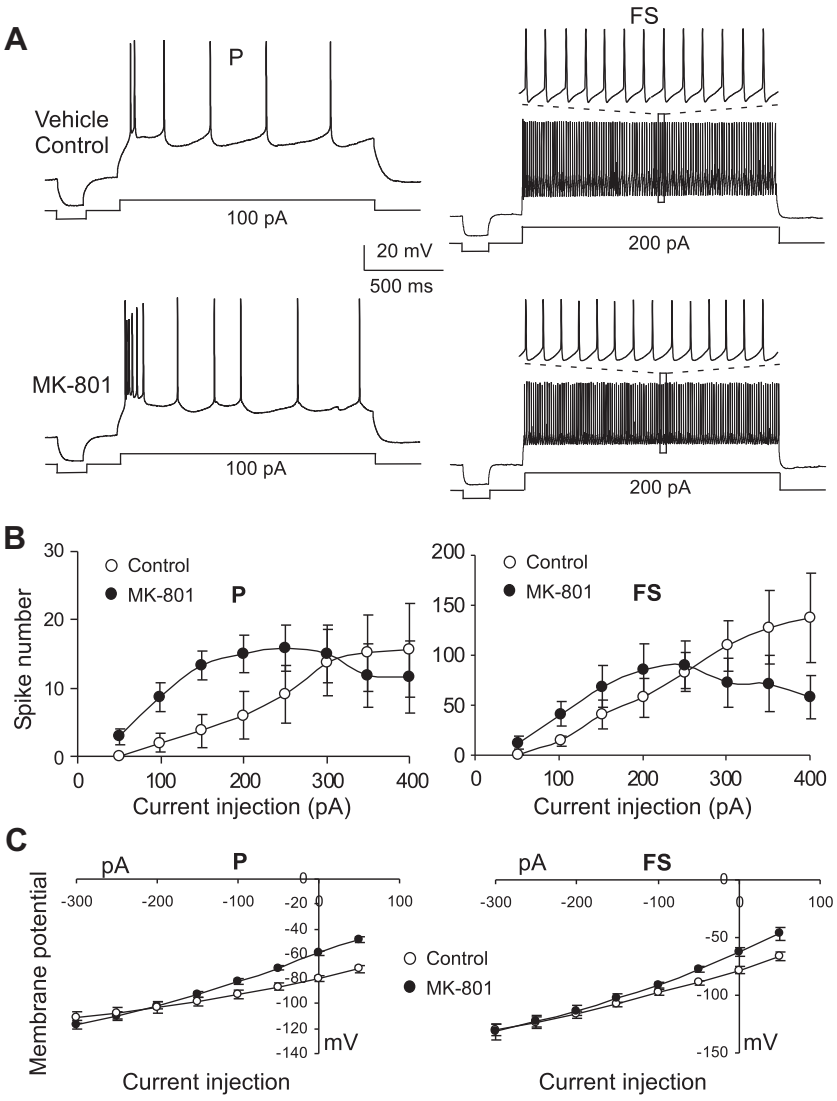

Fig. 1. Both pyramidal cells and FS interneurons were depolarized by treatment with MK-801. A, The firing patterns of pyramidal cells and FS interneurons (dash-lines denote the expanded spikes within the boxed areas) in response to injection of current in the soma. B, The numbers of action potentials in both FS and pyramidal cells were not significantly different overall compared with saline vehicle control groups (two-way ANOVA: $n = 7$; $F = 0.975$, $p = 0.448$ for pyramidal cells; $n = 5$, $F = 1.022$, $p = 0.427$ for FS interneurons). C, The summary graphs show the significant shift of I–V curves toward depolarization induced by MK-801 in both FS and pyramidal cells (P: $n = 7$ for both control and MK-801, $F = 4.764$, $p < 0.001$; FS: n = 5 for both control and MK-801, $F = 2.943$, $p < 0.01$). Overall, pyramidal neurons appeared to be easily excited; FS interneurons were more difficult to study due to rapid rundown during recordings in the MK-801-treated rats. Although MK-801 treatment causes changes in membrane properties in both pyramidal cells and FS interneurons, these neurons are basically healthy and thus the results were comparable. FS, fast-spiking; P, pyramidal.

This raises question of whether single challenge with MK801 also induces changes in intrinsic membrane properties and firing characteristics as exhibited in Table 1. However, as shown in the Supplemental Table 1, single injection of MK-801 did not alter the somatic membrane properties in both pyramidal cells and FS interneurons in the rat PFC despite the significant effects on the axonal terminals (see Supplemental Fig. 1). This result suggests that repeated subchronic treatment with MK-801 induced more severe impairments in the prefrontal neurons than with single injection.

Table 1
Basic properties of pyramidal cells and FS interneurons and in the rat prefrontal cortex.

	Pyramidal cells			FS interneurons		
	Control ($n = 7$)	MK-801 ($n = 7$)	P value	Control ($n = 11$)	MK-801 ($n = 6$)	P value
Resting membrane potential (mV)	−79.6 ± 2.48	−59.5 ± 1.68	<0.01	−78.1 ± 2.68	−62.7 ± 3.50	<0.005
Input resistance (MΩ)	162.6 ± 22.43	217.8 ± 12.42	<0.05	214.9 ± 23.55	211.1 ± 14.41	0.886
Membrane time constant (ms)	16.9 ± 2.35	15.4 ± 2.35	0.309	8.29 ± 0.63	5.14 ± 0.61	<0.005
AP threshold (mV)	−42.5 ± 2.60	−39.1 ± 2.76	0.165	−47.6 ± 1.81	−42.9 ± 4.46	0.512
AP half-width (ms)	2.33 ± 0.29	2.63 ± 0.15	0.312	0.67 ± 0.03	1.22 ± 0.16	<0.005
Fast AHP (mV)	2.04 ± 0.90	2.07 ± 0.82	0.982	16.7 ± 1.68	15.3 ± 1.42	0.608
Late AHP (mV)	12.1 ± 1.12	11.9 ± 1.05	0.883			

AHP, afterhyperpolarization; AP, action potential.

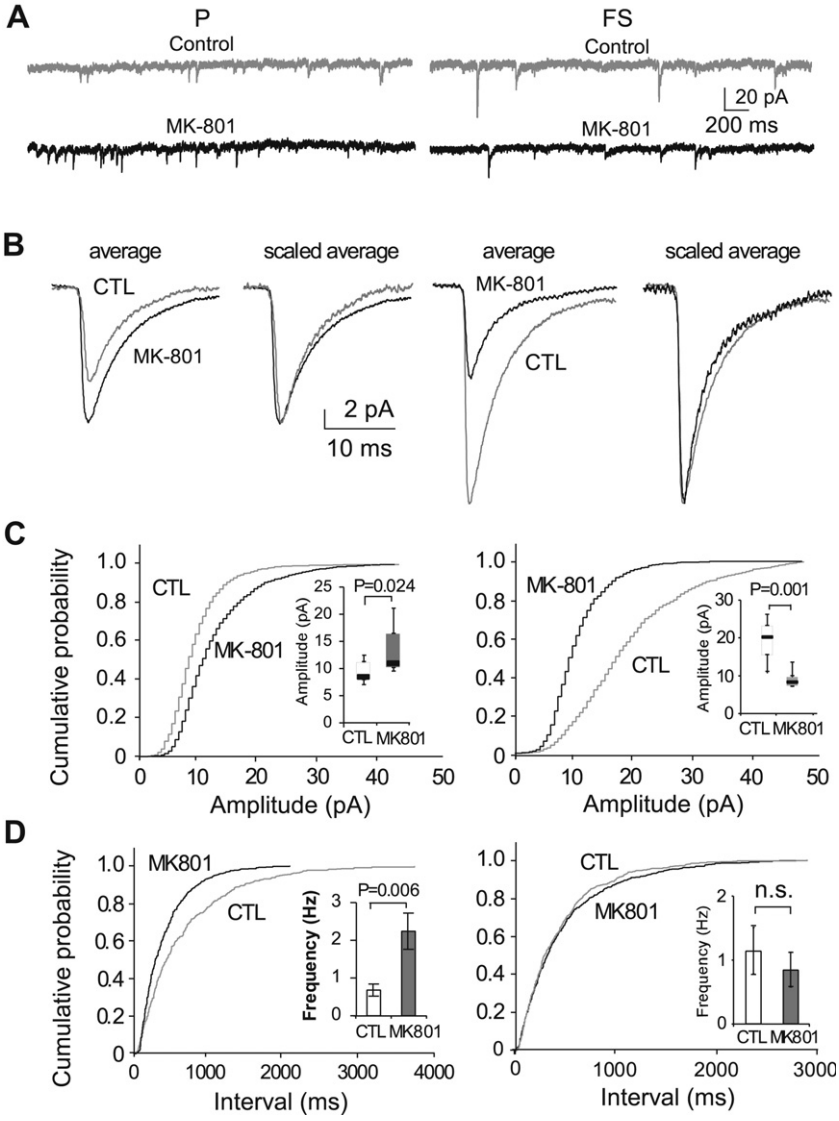

Fig. 2. Prolonged treatment with MK-801 induces opposite homeostatic synaptic scaling of AMPA receptor-mediated mEPSCs in FS interneurons compared with pyramidal cells. A, The sample traces of mEPSC recordings in pyramidal cells and FS interneurons at −70 mV with TTX and PTX in the bath solution. B, Sample traces show that MK-801 induced opposite alterations of mEPSC amplitude in FS and pyramidal cells, with a significant decrease of mEPSC amplitude in FS and an increase in the pyramidal cells. The time constants were, however, unaltered (see scaled traces). C, Cumulative probability histograms show the mEPSC amplitude in the control and the MK-801 model. Insets: average AMPA mEPSCs ($p < 0.001$, $n = 7$ for both control and MK-801 in FS cells; and $p < 0.05$, $n = 9$ both control and MK-801 in pyramidal neurons). D, Cumulative probability histograms of mEPSCs interevent interval from control and the MK-801 model. Insets: average frequency of AMPA mEPSCs. The frequency was unaltered in FS interneurons but was significantly increased in pyramidal cells ($p < 0.01$, $n = 7$).

3.3. New presynaptic NMDA receptors were detected in the glutamatergic terminals targeting pyramidal neurons after MK-801 administration

Presynaptic NMDA receptors enhances glutamate release and thus increases the frequency of mEPSCs in the entorhinal cortex (Berretta and Jones, 1996; Woodhall et al., 2001; Yang et al., 2006), visual cortex (Sjostrom et al., 2003), and dentate gyrus (Jourdain et al., 2007). Although presynaptic NMDA receptors were reported in the entorhinal cortex in adult animals (Woodhall et al., 2001; Yang et al., 2006), it has also been reported that presynaptic NMDA receptors are gradually lost during cortical development (Corlew et al., 2008; Mathew and Hablitz, 2011). Whether presynaptic NMDA receptors exist in axon terminals targeted on pyramidal cells and FS interneurons in the adolescent rat PFC is unknown. In addition, because MK-801 could potentially block both pre- and postsynaptic NMDA receptors in a use- and activity-dependent manner, we tested whether the altered glutamate release and postsynaptic AMPA receptor-mediated mEPSCs were attributable to the changes in presynaptic NMDA receptors in both pyramidal cells and FS interneurons.

We first examined whether presynaptic NMDA receptors exist in the excitatory synaptic terminals connected to layer II/III pyramidal neurons in the mPFC. To examine the presynaptic NMDA receptors, paired-pulse stimulation was employed with interpulse interval of 50 ms (20 Hz) and stimulus frequency at 0.1 Hz (every 10 s). The intracellular solution containing MK-801 (1 mM) was applied to block the postsynaptic NMDA receptors, as described in previous study (Mathew and Hablitz, 2011; Sjostrom et al., 2003). The evoked AMPA receptor-mediated EPSCs were continuously recorded at a holding potential of −70 mV in the presence of PTX (50 μM) and $GABA_B$ receptor antagonist CGP55845 (1 μM, Tocris

Bioscience, Ellisville, MO). The EPSCs were recorded with input resistance and holding current of the neurons being continuously documented. After 10 min baseline recording, AP5 (50 μM) was added into the bathing solution and the neurons were recorded for another 10 min. The data recorded from the second 5 min in both baseline and AP5 were used for analysis and statistic comparison. The paired-pulse ratio (PPR) was determined as the peak amplitudes of EPSC2/EPSC1. As shown in Fig. 3A and B, paired-pulse facilitations were usually observed in the baseline in 11 out of 15 neurons recorded. In contrast, bath application of AP5 significantly decreased the PPR by 26.7 ± 3.56% ($n = 15$, $p < 0.0001$; Fig. 3A,B) and the 2nd EPSC amplitudes without alterations in either input resistance or holding current (Fig. 3A).

To further confirm the existence of presynaptic NMDA receptors, we recorded evoked EPSCs in pyramidal neurons at −70 mV in the presence $GABA_A$ receptor antagonist PTX (50 μM) and AP5 (50 μM) with minimal stimulation. We calculated the percent changes of synaptic failures before and after AP5 application (Fig. 3C). We found that AP5 wash-in significantly decreased the EPSC amplitude from 17.7 ± 4.09 pA to 11.0 ± 2.97 pA ($n = 6$, $p = 0.014$) and increased the synaptic failure from 50.0 ± 7.30% to 72.5 ± 8.64% ($n = 6$, $p = 0.012$; Fig. 3D). These data confirm the presence of presynaptic NMDA receptors in the adolescent rat prefrontal cortical neurons.

Next, we tested whether the mEPSC amplitude changes induced by chronic MK-801 treatment (shown in Fig. 2) were associated with alterations in presynaptic NMDA receptors. The mEPSC recordings were carried out with bath solution containing TTX (1 μM) and PTX (50 μM) in both the vehicle control and MK-801-treated group. The membrane potentials of the pyramidal neurons were clamped at −70 mV during recordings. The mEPSCs were recorded for 10 min as the baseline (pre-AP5), and then AP5 (50 μM) was administered to block presynaptic NMDA receptors; mEPSCs were continuously recorded for another 10 min. The AMPA mEPSCs could act as activity reporters of presynaptic NMDA receptors under this condition because the role of postsynaptic NMDA receptors in the AMPA mEPSCs is negligible under such a condition (Rotaru et al., 2011; Sjostrom et al., 2003). As shown in Fig. 4, we found that the frequencies of the mEPSCs were significantly reduced by application of AP5 in both the controls and the MK-801 model (Fig. 4A), whereas the average amplitudes of mEPSCs were not altered by AP5 administration in the neurons treated with MK-801 ($p > 0.05$, $n = 9$; Fig. 4B, C). K–S analysis of the cumulative probability distributions of interevent interval showed significant increases after treatment with AP5 ($p < 0.001$, $n = 9$ for both vehicle control and MK-801 group; Fig. 4D). Indeed, the average frequency of mEPSCs in the vehicle control decreased from 0.67 ± 0.17 Hz to 0.42 ± 0.12 Hz after AP5 ($p < 0.05$, $n = 9$), whereas the frequency in the MK-801 model decreased from 2.24 ± 0.46 Hz to 0.57 ± 0.15 Hz ($p < 0.01$, $n = 9$; Fig. 4E). It is interesting that both the mEPSC amplitude (9.33 ± 0.67 pA in control vs. 13.11 ± 1.36 pA in MK-801, $n = 9$; $p < 0.05$) and frequency (0.67 ± 0.17 Hz in control vs. 2.24 ± 0.46 Hz in MK-801, $n = 9$; $p < 0.01$) in the MK-801 model were significantly higher than those in the vehicle control under the pre-AP5 conditions (Fig. 4C, E), suggesting an increased presynaptic release and/or postsynaptic AMPA receptor-mediated scaling that are affected by presynaptic NMDA receptors. This conclusion was further supported by displaying the data in histogram which was created with

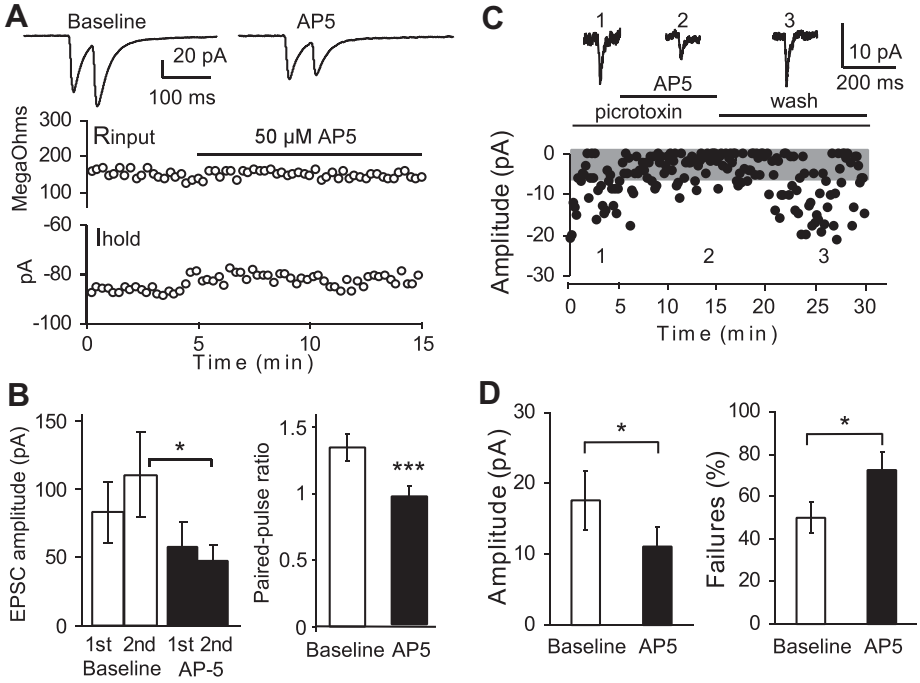

Fig. 3. Presynaptic NMDA receptors exist in excitatory synaptic terminals targeting on pyramidal neurons in adolescent rat PFC. A, The upper panel shows the sample traces of evoked EPSCs with paired-pulse stimuli in baseline and AP5 application. The bottom panel shows the changes of input resistance (Rinput) and holding current (Ihold) in the recorded neuron. The EPSCs were pharmacologically isolated by recording the neuron at −70 mV in the presence of 50 μM picrotoxin, 1 μM CGP55845 to block both $GABA_A$ and $GABA_B$ receptors. The postsynaptic NMDA receptors were blocked with intracellular solution containing 1 mM MK-801. AP5 wash-in clearly altered the amplitude of both first and second EPSCs without affecting the input resistance and holding current. B, Summary histograms showing the effects of AP5 on EPSC amplitudes and paired-pulse ratio (PPR). Most of the neurons exhibited paired-pulse facilitation with the amplitudes of the second EPSCs larger than the first ones in the baseline recordings, while wash-in of AP5 (50 μM) significantly decreased PPR ($n = 15$, $p < 0.0001$). C, The effects of AP5 on eEPSCs induced with minimal stimulation which produced about 50% synaptic failures in all stimulus trials. The evoked EPSCs in pyramidal neurons were recorded in the presence of $GABA_A$ receptor antagonist picrotoxin (50 μM) and AP5 (50 μM) with minimal stimulus. The upper panel shows the average sample traces of AMPA receptor-mediated EPSCs in baseline (1), AP5 (2), and washout (3). Lower panel: plot of eEPSC amplitudes versus recording time shows the depressive AP5 effect on the minimal eEPSCs in a pyramidal cell. Shadow area denotes synaptic failure which was determined as same or below 3 times mean root square of the baseline under our recording condition. D, Summary histograms exhibited that AP5 application significantly decreased the EPSC amplitude ($n = 6$; $p = 0.001$) but increased the failure rate ($n = 6$; $p = 0.012$). These results further confirm the existence of presynaptic NMDA receptors in the axon terminals targeting pyramidal neurons in the adolescent PFC.

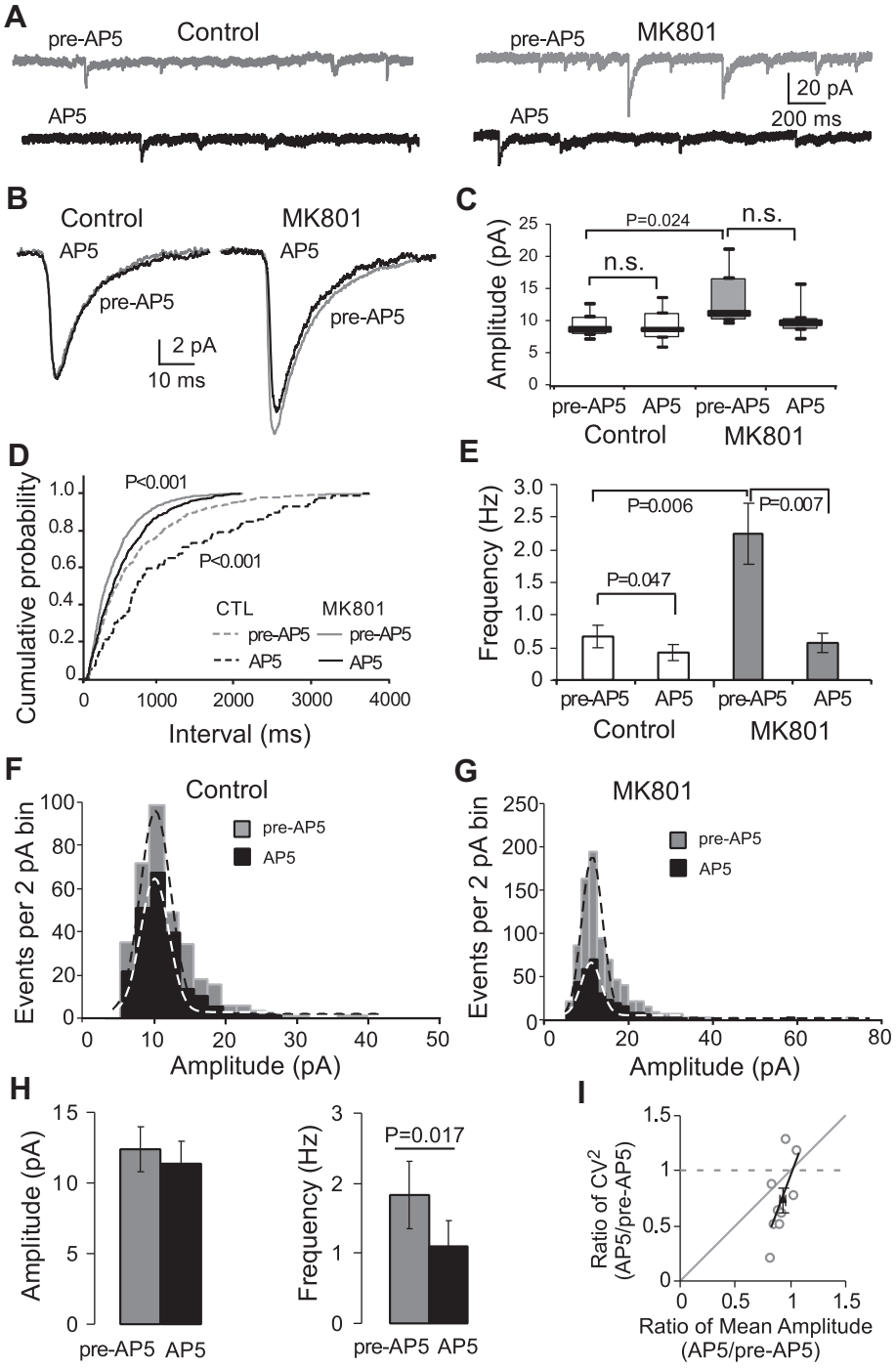

Fig. 4. Treatment with MK-801 induces new insertions of NMDA receptors to the presynaptic terminals targeting pyramidal cells. A, Sample traces of AMPA mEPSCs in pre-AP5 and AP5 in the pyramidal neurons. B, Average mEPSCs before and after application of AP5. C Although mEPSC amplitude was significantly increased in the MK-801 model compared with the control, it was not affected by AP5 in either the control or the MK-801 treatment group. D, Cumulative probability of mEPSC interevent interval before and after application of AP5. E, In contrast to the unaltered frequency in FS interneurons in the MK-801 model, the frequencies of AMPA mEPSCs in pyramidal cells were significantly reduced by AP5 in both the control and the MK-801 groups. The frequency was significantly increased 3-fold in MK-801 compared with the control. F, G, The histograms show that mEPSC events on pyramidal cells were significantly decreased by application of AP5 in both control and MK-801 groups. H, The mEPSC amplitude was not affected by AP5 application ($n = 9$, $p = 0.119$) but the mEPSC frequency was significantly decreased by AP5 ($n = 9$, $p = 0.017$) when postsynaptic NMDA receptors were blocked with 1 mM MK-801 loaded into the pipette solution. I, CV analysis of the mEPSCs projected a reduced presynaptic release probability after AP5 application because the ratio (AP5/pre-AP5) of CV^2 was located under 45° line. The trend of changes was similar to those exhibited in Fig. 4C, E and G. These data indicated that MK-801 treatment induced new additions of presynaptic NMDA receptors to the glutamatergic axon terminals targeting pyramidal neurons.

the mEPSC event numbers at a bin size of 2 pA. The amplitude histograms of mEPSC events also exhibited significant decreases by AP5 administration in both vehicle control and MK-801 model (Fig. 4F, G). These data indicated that MK-801 treatment induced new additions of presynaptic NMDA receptors to the axon terminals targeting the pyramidal neurons.

Previous study indicated that MK-801 reached maximal concentrations in plasma and brain within 10–30 min of injection

(2 mg/kg) with an elimination half-life of 1.9 and 2.05 h, respectively (Vezzani et al., 1989). Because concentration-time curve in brain area is significantly longer than the concentration–time curve in plasma (Vezzani et al., 1989), it is possible that there is remaining MK-801 which might be still effective but not sufficient to block all NMDA receptors after 24 h. To confirm the presence and function of presynaptic NMDA receptors and to exclude the effects of postsynaptic NMDA receptors, we recorded mEPSCs in pyramidal cells with 1 mM MK-801 loaded into pipette solution in subchronic MK-801-treated rats. We found that mEPSC amplitude was not affected by AP5 application (12.4 ± 1.58 pA for pre-AP5 versus 11.4 ± 1.50 pA for AP5; $n = 9$, $p = 0.119$) but the mEPSC frequency was significantly decreased by AP5 (1.83 ± 0.48 Hz for pre-AP5 versus 1.11 ± 0.38 Hz for AP5; $n = 9$, $p = 0.017$; Fig. 4H). Further analysis with coefficient of variation (CV) of the mEPSCs projected a reduced CV after AP5 application because the average ratio (AP5/pre-AP5) of CV^2 was about 0.7 and located below the horizontal line in the lower half quadrant in the MK-801 model (Fig. 4I) (Gray et al., 2011). This analysis indicates that AP5 directly reduced the presynaptic release probability as postsynaptic NMDA receptors were blocked by loaded MK-801 loaded in the pipette. The trend of changes was similar to those exhibited in Fig. 4C, E and G. These data confirmed MK-801's effect on the newly inserted presynaptic NMDA receptors in the glutamatergic terminals targeting pyramidal neurons.

3.4. Presynaptic NMDA receptors in the axonal terminals targeting FS interneurons were blocked without new insertion in MK-801 model

Whether presynaptic NMDA receptors exist in axon terminals targeted on FS interneurons in the PFC is unknown. Thus, we

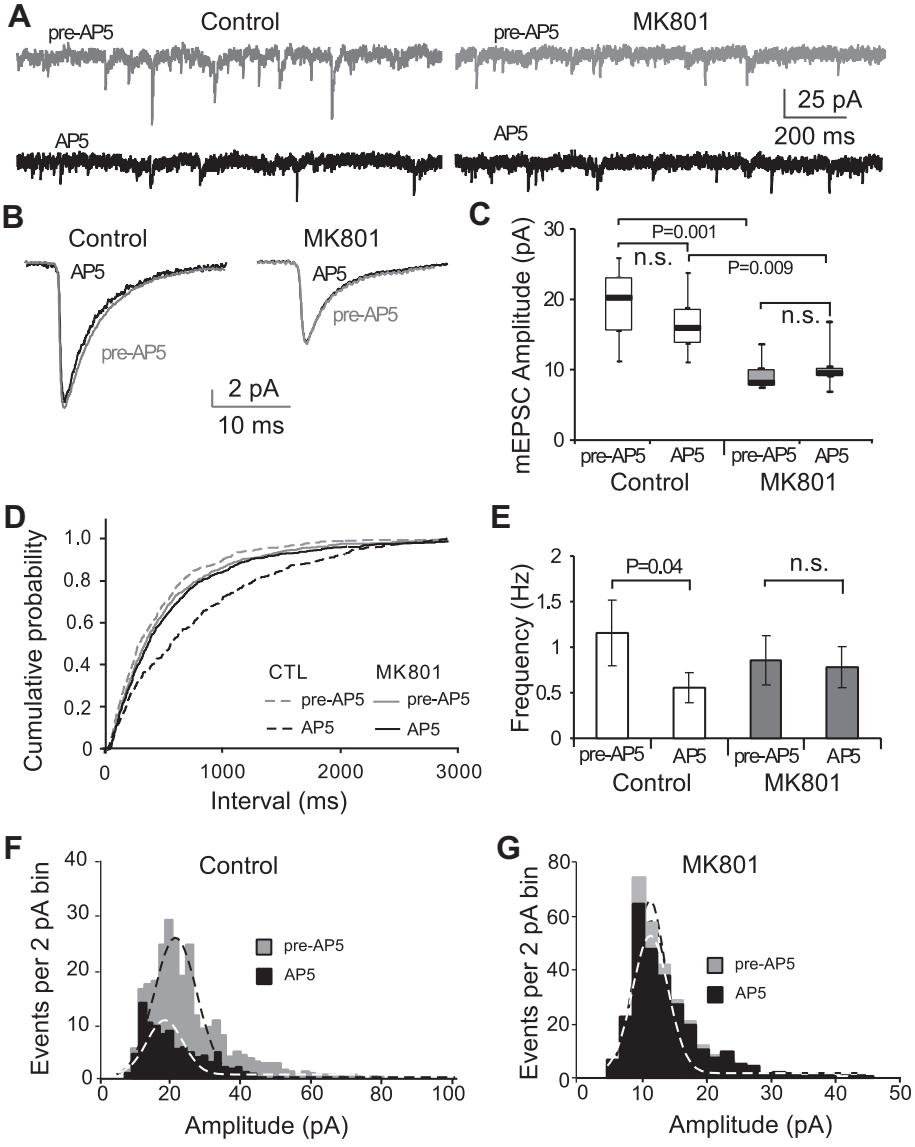

Fig. 5. Presynaptic NMDA receptors in the axon terminals targeting FS interneurons were completely blocked by treatment with MK-801. A, Sample traces of mEPSCs in pre-AP5 and AP5 applications from saline control and MK-801-treated animals. The mEPSCs were recorded at −70 mV in the presence of PTX and TTX. B, Average mEPSCs from both pre-AP5 and AP5 applications showing a significantly reduced mEPSC amplitude and unaltered kinetics in the MK-801 model. C, Summary histogram shows that despite the significantly smaller mEPSC amplitude in the MK-801-treated group compared with the saline vehicle control, the mEPSC amplitudes in the FS interneurons were not affected by application of AP5 in either the control or the MK-801-treated group. D, Cumulative probability of mEPSC interevent intervals in pre-AP5 and AP5 applications. E, Frequency histograms in pre-AP5 and AP5 applications from control and the MK-801 model show that the mEPSC frequency was significantly reduced by AP5 in the control but not in the MK-801-treated group. F, G, The histograms show that mEPSC events were significantly decreased by AP5 administration in vehicle control but not in MK-801 model. n.s., not significant.

examined the presynaptic NMDA receptors on FS interneurons. Similarly, the membrane potentials of the FS interneurons were clamped at −70 mV during recordings and the bath solution contained TTX (1 μM) and PTX (50 μM). The mEPSCs were recorded for 10 min as the baseline (pre-AP5), followed by AP5 (50 μM) administration for another 10 min. In the cells recorded in the vehicle control rats, the average amplitude of mEPSCs exhibited no significant changes in response to AP5 administration ($p > 0.05$, $n = 7$; Fig. 5A–C), whereas the interevent interval increased significantly ($p < 0.0001$, $n = 7$), indicating a significant decrease of the mEPSC frequency (1.17 ± 0.37 Hz in pre-AP5 vs. 0.56 ± 0.16 Hz in AP5, $n = 7$, $p < 0.05$; Fig. 5D, E).As exhibited in Fig. 5F, the mEPSCs events were markedly decreased by AP5 administration in the neurons recorded from the vehicle control. These data suggested that presynaptic NMDA receptors are still distributed in the glutamatergic axon terminals targeted on FS interneurons in the normal adolescent rat PFC. In contrast to the vehicle control, neither the average amplitude ($p > 0.05$, $n = 7$) nor the frequency ($p > 0.05$, $n = 7$) of the mEPSCs between pre-AP5 and AP5 treatment was changed in the MK-801 model (Fig. 5B, C, E, G). These data indicate that MK-801 blocked the existing presynaptic NMDA receptors in the axon terminals targeted on FS interneurons and that the blockade was long-lasting without recovery and new insertion even after 24 h of MK-801 administration.

We further identified the subunit components of NMDA receptors in the presynaptic axonal terminals targeting on pyramidal cells and FS interneurons 24 h after a single injection of MK-801 (0.1 mg/kg, i.p.) in four rats (aged PD40-44, $n = 4$). As shown in Supplemental Fig. 2, we recorded mEPSCs in both pyramidal neurons ($n = 7$) and FS interneurons ($n = 6$) with intracelluar solution containing 1 mM MK-801 (at a holding potential of −70 mV) in the presence of 50 μM PTX and 1 μM TTX. We found that ifenprodil had no effects on the mEPSC amplitudes in both pyramidal cells ($p = 0.127$) and FS interneurons ($p = 0.432$). However, ifenprodil significantly decreased mEPSC frequency by 33.8 ± 0.08% in pyramidal cells ($p = 0.003$) but had no clear effects on the mEPSC frequency in FS interneurons ($p = 0.543$). These results suggest that the NMDA receptor subunits in the presynaptic axonal terminals targeting on pyramidal cells and FS interneurons appear to be different, with more NR2B subunit in the axon terminals targeting on pyramidal cells than those on FS interneurons.

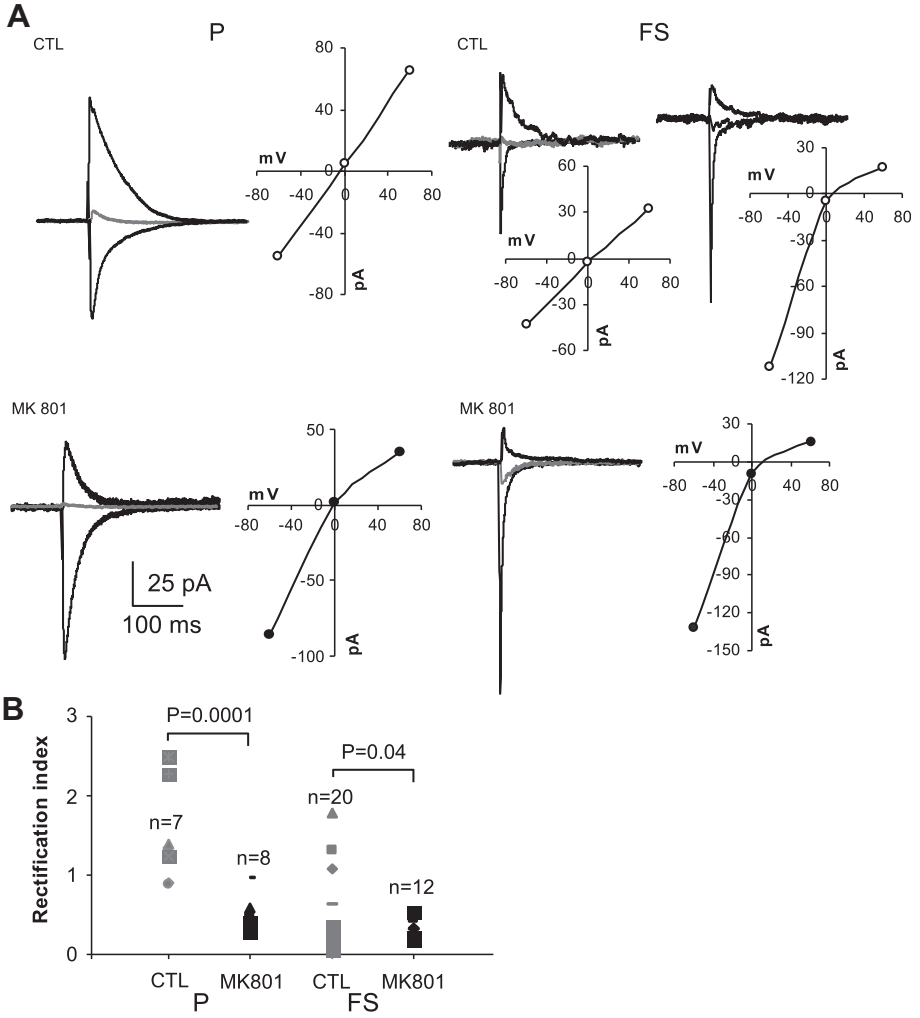

Fig. 6. MK-801 treatment induced dramatic changes in AMPA receptor-mediated current in both pyramidal cells and FS interneurons. A, Sample traces and I–V curves showing the rectification of AMPA receptor-mediated EPSCs recorded at −60, 0, and +60 mV, respectively, in the presence of AP5 and PTX. The AMPA-EPSCs in FS interneurons exhibited both large and small RIs whereas those in pyramidal neurons displayed large RIs only in the vehicle control rats. Dramatic decreases in RIs were observed in both pyramidal cells and FS interneurons in MK-801-treated rats. B, Summary scatter plot shows that RIs in both pyramidal cells and FS interneurons were significantly decreased in the MK-801 model compared with controls.

3.5. Increased CP-AMPA receptors in both pyramidal cells and FS interneurons in MK-801 model

The calcium (Ca^{2+}) permeability of AMPARs is critically dependent on GluR2 subunits. Those containing GluR2 are Ca^{2+} impermeable (CI-AMPAR) and have a linear current–voltage (I–V) relation, and those lacking GluR2 are Ca^{2+} permeable (CP-AMPAR) and are strongly inwardly rectifying (Hollmann and Heinemann, 1994; Jonas and Burnashev, 1995; Wang and Gao, 2010). GluR2-lacking CP-AMPARs have recently received considerable attention because of their postulated role in synaptic plasticity (Adesnik and Nicoll, 2007; Clem and Barth, 2006; Liu and Cull-Candy, 2000; Plant et al., 2006) and neurological disorders (Cull-Candy et al., 2006; Isaac et al., 2007; Liu et al., 2006; Liu and Zukin, 2007; Tanaka et al., 2000). We recently reported that most of the FS interneurons in the PFC express calcium-permeable AMPA receptors (CP-AMPA) during the adolescent period (Wang and Gao, 2010). We therefore wondered whether MK-801 treatment also affects the calcium permeability of AMPA receptors. To explore the changes in AMPA receptors in the MK-801 model, we plotted the I–V curve of the AMPA receptor-mediated EPSCs in both pyramidal neurons and FS interneurons. The AMPA receptor-mediated currents were recorded at membrane potentials held at −60, 0, and +60 mV, respectively. The recorded neurons were bathed with normal Ringer's solution containing PTX (50 μM) and AP5 (50 μM), with spermine (50 μM) included in the intracellular solution (Wang and Gao, 2010). The currents were evoked by low-intensity stimulation of intracortical fibers with a bipolar electrode placed near the soma in layer II–III. The I–V curves of AMPA-EPSCs in the FS interneuron and pyramidal cells are shown in Fig. 6A, and the RIs of EPSCs were calculated by the conductance ratios of $EPSC_{+60\ mV}/EPSC_{-60\ mV}$. It is known that cells expressing appreciable levels of GluR2, which form calcium-impermeable (CI)-AMPA receptors with linear or outwardly rectifying I–V relationships, were usually unaffected by the application of spermine. As reported in our recent study, the breakup value of RI was set at 0.7; the recorded pyramidal neurons and FS interneurons in the PFC of the vehicle control and the MK-801 can easily be identified as cells with CI- or CP-AMPA receptors. All of the pyramidal neurons examined ($n = 7$) showed as CI in the PFC in normal adolescent rats, consistent with the findings from a previous study (Kumar et al., 2002), whereas in the MK-801 model, the RI values in 7 of 8 pyramidal cells recorded were lower than 0.7, significantly deceased compared with those in the vehicle controls (RI = 1.50 ± 0.24 in control vs 0.47 ± 0.08 in MK-801, $\chi^2 = 28.02$, $p < 0.001$; Fig. 6A, B). Similarly, in the 20 FS interneurons recorded in the vehicle control, 14.3% primarily expressed CI- and 85.7% expressed CP-AMPA receptors, consistent with our recent report (Wang and Gao, 2010). In contrast, in the MK-801 model, all of the 12 FS interneurons tested exhibited CP-AMPA receptors. The RIs were significantly decreased in the MK-801-treated group compared with those in vehicle controls (RI = 0.36 ± 0.10 in control vs 0.30 ± 0.04 in MK-801, $\chi^2 = 22.71$, $p < 0.0001$, Fig. 6A, B). These results suggest that treatment with MK-801 induced clear changes in the AMPA receptor subunits and increased the CP-AMPA receptors in both pyramidal neurons and FS interneurons.

To confirm this finding, we conducted an additional experiment to examine the amplitude changes of AMPA receptor-mediated currents with bath application of CP-AMPARs antagonist NASPM in MK-801 model. The recorded neurons were clamped at −70 mV and bathed with 50 μM PTX and 50 μM AP5 to isolate AMPA currents. After the currents were stable, we recorded 5 min as baseline and then recorded another 10 min with additions of 50 μM NASPM, followed by washing out with ACSF containing PTX and AP5. In the normal control animals, the EPSC amplitude was not altered with NASPM application in pyramidal cells ($n = 9$, $p = 0.240$) but was significantly decreased by 20.8% in FS interneurons ($n = 6$, $p = 0.036$; Fig. 7A and B). These results suggested that FS interneurons, but not pyramidal cells, express CP-AMPA receptors in normal rat PFC during adolescent period, in support of our recent report (Wang and Gao, 2010). In contrast, in the MK-801 model, NASPM application significantly reduced the EPSC amplitude by more than 30% in pyramidal cells ($n = 6$, $p = 0.0014$) and about 42% in FS interneurons ($n = 6$, $p = 0.011$; Fig. 7A and B). The percent changes of EPSC amplitude in MK-801-treated neurons were significantly higher than those in the control for both pyramidal neurons and FS interneurons ($p = 0.048$ for pyramidal cells and $p = 0.048$ for FS interneurons). These results further confirmed that CP-AMPA receptors were indeed increased in both pyramidal neurons and FS interneurons in the MK-801-treated animals.

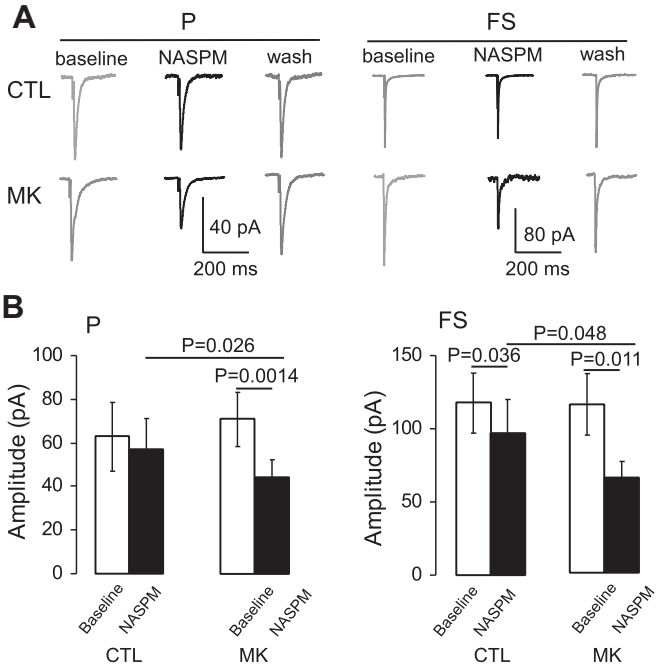

Fig. 7. MK-801 increased CP-AMPA receptor expressions in both pyramidal cells and FS interneurons in adolescent rat PFC. A, The sample traces of AMPA-EPSCs in baseline, NASPM administration, and washout for pyramidal cells and FS interneuron under both conditions of normal control and MK-801 model. The recorded neurons were clamped at −70 mV and bathed with 50 μM PTX and 50 μM AP5 to isolate AMPA currents. After the currents were stable, we recorded 5 min as baseline and then recorded another 10 min with wash-in of 50 μM NASPM, followed by washing out with ACSF containing PTX and AP5. B. Summary histograms of EPSC amplitude changes with NASPM administration. In the normal control animals, the EPSC amplitude was not altered by NASPM in pyramidal cells ($n = 9$, $p = 0.240$) but was significantly decreased by 20.8% in FS interneurons ($n = 6$, $p = 0.036$). In contrast, in the MK-801 model, NASPM significantly reduced the EPSC amplitude by more than 30% in pyramidal cells ($n = 6$, $p = 0.0014$) and about 42% in FS interneurons ($n = 6$, $p = 0.011$). The percent changes of EPSC amplitude in MK-801-treated neurons were significantly higher than those in the control for both pyramidal neurons and FS interneurons ($p = 0.048$ for pyramidal cells and $p = 0.048$ for FS interneurons), suggesting that CP-AMPA receptors were indeed increased in both pyramidal neurons and FS interneurons in the MK-801-treated animals.

3.6. Differences in Ca^{2+} permeability of AMPA receptors in synapses on pyramidal cells and FS interneurons in the MK-801 model

The significant increases in the CP-AMPA in both pyramidal neurons and FS interneurons in the MK-801-treated rats suggest a possible increase in Ca^{2+} permeability. To explore this possibility in the MK-801 model, we measured the peak amplitude values of AMPA-receptor-mediated EPSCs with neurons clamped at −60, 0, and +60 mV, respectively, as described above. The I–V relationships

for synaptic responses of AMPA receptors were first recorded in normal Ringer's solution (2 mM Ca^{2+}) in the presence of PTX (50 μM); then the Ringer's solution was replaced with a solution high in Ca^{2+} (30 mM). We found that after bathing with a 30-mM Ca^{2+} solution for 10 min, the RI values of the FS interneurons in the MK-801 model also decreased significantly ($p < 0.05$, $n = 7$), whereas the RI values in pyramidal cells were not significant from those in the control ($p > 0.05$, $n = 5$; Fig. 8A, B). These data suggested that the synaptic AMPA receptors in the FS interneurons (but not in the pyramidal neurons) in the MK-801 model were altered to be more permeable to Ca^{2+} influx, which may increase the vulnerability of FS interneurons to harmful stimulation.

4. Discussion

To test the synaptic mechanisms of the NMDA hypofunction hypothesis for schizophrenia, we selectively examined the functional changes in AMPA and NMDA receptors in individually identified FS interneurons compared with those in pyramidal cells in the MK-801 model of adolescent rat PFC. We found that, although both pyramidal neurons and FS interneurons were impaired by a subchronic blockade of NMDA receptors, MK-801 induced several selective alterations in FS interneurons but not in pyramidal cells, including a significantly reduced RI and increased calcium permeability. In particular, MK-801 induced a significant decrease in the AMPA-mEPSC amplitude in FS interneurons but significant increase in both frequency and amplitude of AMPA-mEPSC in pyramidal neurons. In addition, presynaptic NMDA receptors in the axon terminals targeting FS interneurons were completely blocked without recovery whereas new presynaptic NMDA receptors were inserted into the glutamatergic terminals onto pyramidal neurons.

Numerous studies have reported NMDA receptor changes induced by administration of NMDA antagonists (Barbon et al., 2007; Gao and Tamminga, 1995; Harris et al., 2003; Lindahl and Keifer, 2004; Rujescu et al., 2006; Wang et al., 1999). There is, however, little consensus on the alterations in NMDA receptor subunits that might contribute to the NMDA hypofunction on the basis of the observations obtained from postmortem brains in patients with schizophrenia (Akbarian et al., 1996; Luthi et al., 2001; Matthews et al., 2000; Meador-Woodruff and Healy, 2000; Moghaddam, 2003). Nevertheless, these studies have provided strong evidence for the network effects of these compounds and established the theoretical basis for the NMDA hypofunction hypothesis for schizophrenia pathogenesis (Lisman et al., 2008). Despite the intriguing hypothesis of disinhibition based on the deficits of GABAergic transmission in the brains of patients with schizophrenia, little evidence is available showing how NMDA receptor antagonist affects the synaptic transmissions on the identified FS interneurons and how the alteration in synaptic transmission mechanistically elucidates the dysfunction of the network. We found that the AMPA mEPSCs were significantly and distinctly changed in the pyramidal cells and FS interneurons by the subchronic in vivo blockade of NMDA receptors with MK-801. Our data indicate that MK-801 induced the hypofunction of FS interneurons by both pre- and postsynaptic mechanisms. The increased amplitude of AMPA mEPSCs in the pyramidal neurons that we observed is consistent with the findings from several previous studies conducted in cultured hippocampal and cortical neurons

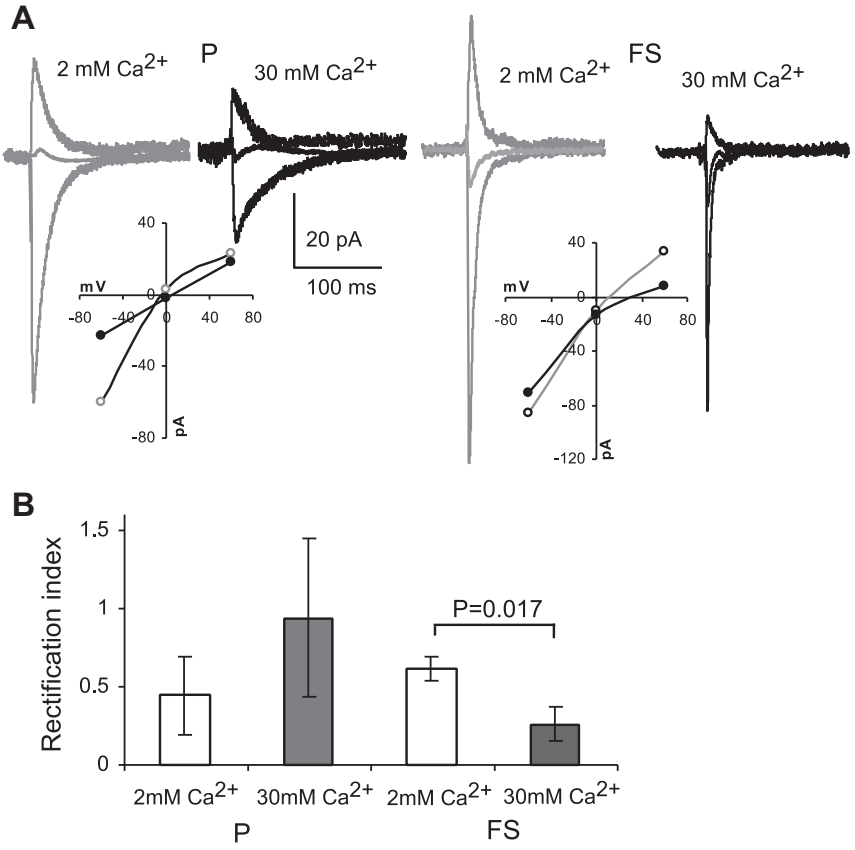

Fig. 8. Differential Ca^{2+} permeability of synaptic AMPA receptors in pyramidal cells and FS interneurons in the MK-801 model. A, Representative traces (at −60, 0, and +60 mV) from the administration of control (2 mM) and highly concentrated Ca^{2+} (30 mM) solutions in pyramidal cells and FS interneurons in the MK-801-treated rats. B, The RIs of FS interneurons but not of pyramidal neurons were significantly decreased by administration of highly concentrated Ca^{2+} solution ($p < 0.05$, $n = 7$), indicating a differential calcium permeability in FS interneurons versus pyramidal neurons.

(Kato et al., 2007; Sutton et al., 2006, 2004; Turrigiano and Nelson, 2004). These studies showed that postsynaptic scaling in AMPA mEPSCs occurs after chronic blockade of NMDA receptors with AP5 (Kato et al., 2007; Sutton et al., 2006) or of neuronal activity with TTX (Sutton et al., 2006, 2004; Turrigiano and Nelson, 2004). In addition, the frequency of AMPA mEPSCs in pyramidal neurons (but not FS interneurons) was significantly increased. In contrast, the amplitude (but not frequency) of AMPA mEPSCs in FS interneurons was significantly decreased, distinctly different from the changes in pyramidal neurons. Altogether, our data provide novel evidence of cell-type specific alterations of AMPA receptors, i.e., decreased excitatory response in FS interneurons (reduced AMPA current) and increased glutamate release and response in pyramidal neurons (increased frequency and amplitude of AMPA current). How could these happen? Target-specific expression of pre- and postsynaptic mechanisms of synaptic transmission has been shown in a variety of central neurons under by a number of laboratories (Bacci et al., 2005; Isaac et al., 2007; Toth and McBain, 2000), including our own studies in dopaminergic regulation (Gao and Goldman-Rakic, 2003; Gao et al., 2003) and MK-801 effects on NMDA receptors (Xi et al., 2009b). These data have demonstrated that synaptic transmission between single axons diverging onto distinct target neurons can behave independently, differentially influencing activity in the target neuron (Toth and McBain, 2000).

In addition, our data indicate that presynaptic NMDA receptors remain available in the axon terminals targeting on PFC layer II/III pyramidal neurons and FS interneurons in the normal adolescent animals, differing from previous studies reported in primary visual cortex (Corlew et al., 2008, 2007) but were consistent with those findings in entorhinal cortex (Woodhall et al., 2001; Yang et al., 2006). Our data thus suggest a brain region difference for the development of presynaptic NMDA receptors. Presynaptic NMDA receptors are known to be critical to facilitate glutamate release and alterations of presynaptic receptors dramatically affect the neuronal and network activity (Bidoret et al., 2009; Brasier and Feldman, 2008; Corlew et al., 2008, 2007; Rodriguez-Moreno and Paulsen, 2008). Indeed, we found that presynaptic NMDA receptors in the synaptic inputs onto pyramidal neurons and FS interneurons were differentially affected by MK-801 treatment, further support a cell-type specific action of MK-801. In the excitatory axonal terminals targeting onto FS interneurons, presynaptic NMDA receptors were completely blocked without recovery. In contrast, more presynaptic NMDA receptors were detected in the glutamatergic axonal terminals targeting pyramidal neurons. The blockade of presynaptic NMDA receptors in the excitatory inputs onto FS interneurons could significantly reduce glutamate release in the synapses distributed on these cells and thus cause a reduced inhibition because AMPA receptors play a critical role in the regulation of FS interneuron functions (Rotaru et al., 2011). The reduced excitatory drive in FS interneurons and the increased excitation in pyramidal neurons will consequently change the excitation–inhibition balance, which could eventually cause over-excitation in pyramidal neurons, excessive glutamate release, and even excitotoxicity in the limbic regions, as proposed in the hypothesis of NMDA hypofunction (Lisman et al., 2008; Olney and Farber, 1995).

Another major finding in our study is the differential changes in postsynaptic AMPA receptors on FS interneurons and pyramidal cells. We found that in the MK-801 model, the RIs in both pyramidal neurons and FS interneurons were significantly decreased, indicating a clear change in AMPA receptor subunits (i.e., GluR1 or GluR2). This result was consistent with that of a previous study in cultured neurons in which NMDA blockade enhanced the synthesis and surface expression of GluR1 but not of GluR2 subunits (Sutton et al., 2006). It is possible that a high concentration of glutamate accumulated in the synaptic cleft lead to the downregulation of GluR2 (Barbon et al., 2007) or upregulation of GluR1 (Sutton et al., 2006). Although it remains unclear whether the changes in RIs induced by treatment with MK-801 in vivo were attributable to the alteration of GluR1 or GluR2 subunits, the decreases in RIs suggest an increase of CP-AMPA receptors and possibly the increase of calcium permeability as well. Indeed, our data suggest that the calcium permeability significantly increased in FS interneurons. Further biochemical study is needed to determine the changes in AMPA receptor subunits and the associated mechanisms on how blockade of NMDA receptors induces the changes in AMPA receptor subunits. Nevertheless, because of the distinct physiological properties, FS interneurons expressing CP-AMPARs may play different roles in integrating neuronal information within the prefrontal cortical circuit during normal and pathophysiological activities (Isaac et al., 2007; Wang and Gao, 2010). They also show distinct vulnerability to disruptive influences such as NMDA receptor antagonists and other agents associated with Ca^{2+} influx due to high Ca^{2+} permeability. Therefore, the MK-801-induced increase of CP-AMPARs in FS interneurons may make these cells particularly vulnerable to disruptive influences in the PFC, which may explain the selective impairment of GABAergic interneurons, particularly FS interneurons in the MK-801 (Paulson et al., 2003; Simpson et al., 2010) and PCP model (Pratt et al., 2008; Schroeder et al., 2000).

An important question is whether the consequences of repeated dosing with NMDAR antagonists offer a model of schizophrenia with validity and/or practical utility. Numerous studies indicated that, in the rodent and monkey PFC, repeated rather than acute treatment with NMDAR antagonists resembles the neuroanatomical and neurochemical changes observed in schizophrenics more closely (Abdul-Monim et al., 2006; Amitai and Markou, 2009; Amitai et al., 2007; Ashby et al., 2010; Cochran et al., 2003; Enomoto et al., 2005; Hajszan et al., 2006; Jentsch et al., 1997a, 1997b; Mandillo et al., 2003; Murai et al., 2007; Paulson et al., 2003; Rasmussen et al., 2007; Reynolds et al., 2004; Rujescu et al., 2006; Simpson et al., 2010), although not all work is in agreement here (Cochran et al., 2003; van Elst et al., 2005). Our recent studies have also suggested that subchronic administration of MK-801 induces significant alterations in NMDA receptors (Xi et al., 2009b) and these changes can be efficiently reversed by treatment with group II mGluR 2/3 agonist (Xi et al., 2011). However, it should be noted that although subchronic administration of NMDA receptor antagonists are more effective than acute application, adverse effects in this treatment are also obvious. In the present study, we observed significant changes in the passive membrane properties (Table 1). These are probably corresponding to physiological changes in the synapses or ion channels distributed in the neuronal dendrites, as previous study reported (Hajszan et al., 2006). It is also possible that these changes are derived from the drug's excitotoxic effects. For example, subchronic MK-801 injection caused a large depolarizing shift in membrane potential, a decrease in the membrane time constant, and an increase in the action potential half-width in FS interneurons. These changes suggest that ionic channels open at rest and during action potential generation are different between control and MK-801 injected animals. A decrease in the membrane time constant is consistent with a possible dendritic degeneration (Isokawa, 1997). Also, the significant decrease in mEPSC amplitudes in FS interneurons (Fig. 2) suggested a possible loss in the number of synapses (Hajszan et al., 2006). These changes could explain the impaired cognitive deficits induced by low-dose subchronic treatment with MK-801 in adolescent rats (Li et al., 2011) and thus support the validity of the subchronic MK-801 model in addressing important issues in the schizophrenia research (Gilmour et al., 2011).

In summary, our data suggest a distinct cell-type specific and homeostatic synaptic scaling and redistribution of AMPA and

NMDA receptors in response to the subchronic blockade of NMDA receptors and provide a direct mechanistic explanation for the NMDA hypofunction hypothesis that have long been proposed in the NMDA receptor antagonist model.

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Conflict of interest

The authors claim no conflicts.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuropharm.2011.11.024.

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