Prenatal Cocaine Reduces AMPA Receptor Synaptic Expression through Hyperphosphorylation of the Synaptic Anchoring Protein GRIP

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Prenatal cocaine exposure produces sustained neurobehavioral and brain synaptic changes closely resembling those of animals with defective AMPA receptors (AMPARs). We hypothesized that prenatal cocaine exposure attenuates AMPAR signaling by interfering with AMPAR synaptic targeting. AMPAR function is governed by receptor cycling on and off the synaptic membrane through its interaction with glutamate receptor-interacting protein (GRIP), a PDZ domain protein that is regulated by reversible phosphorylation. Our results show that prenatal cocaine exposure markedly reduces AMPAR synaptic targeting and attenuates AMPAR-mediated synaptic long-term depression in the frontal cortex of 21-d-old rats. This cocaine effect is the result of reduced GRIP–AMPAR interaction caused by persistent phosphorylation of GRIP by protein kinase C (PKC) and Src tyrosine kinase. These data support the restoration of AMPAR activation via suppressing excessive PKC-mediated GRIP phosphorylation as a novel therapeutic approach to treat the neurobehavioral consequences of prenatal cocaine.

Introduction

Prenatal cocaine exposure produces long-lasting detrimental effects on cognitive function, especially evident in learning in both humans and animal models (Romano and Harvey, 1996; Morrow et al., 2006). Although the molecular mechanisms underlying these changes remain elusive, brain glutamate receptors that establish and stabilize synaptic connections during early development are intimately involved. In the adult brain, cocaine profoundly disrupts glutamatergic synaptic transmission and plasticity, in part by dysregulating AMPA receptors (AMPARs) (Wolf et al., 2004; Kauer and Malenka, 2007). A single intravenous cocaine injection to adult rats results in a higher ratio of AMPA- to NMDA-mediated activity changes in the ventral tegmental area and nucleus accumbens (NAc), further illustrating that AMPARs are highly sensitive to cocaine (Ungless et al., 2001; Saal et al., 2003). Extinction training after cocaine self-administration increases AMPAR expression in NAc of adult rats, and NAc overexpression of AMPAR facilitates extinction training (Sutton et al., 2003). Additionally, withdrawal from repeated cocaine expo-

sure upregulates excitatory synapses, whereas cocaine exposure during the withdrawal period initiates synaptic depression in the NAc (Thomas et al., 2001; Boudreau and Wolf, 2005; Boudreau et al., 2007; Kourrich et al., 2007; Conrad et al., 2008). These reports imply that AMPARs are pivotal regulators of cocaine-induced synaptic plasticity underlying altered cognitive processing including drug-seeking behaviors and predict that cocaine exposure during gestation may profoundly influence AMPARs.

AMPA-mediated fast excitatory synaptic transmission in brain modulates neuronal development and maturation. AMPARs are composed of hetero-oligomeric combinations of GluR1–R4 subunits (Hollmann and Heinemann, 1994). The synaptic trafficking of AMPARs regulates activity-dependent synaptic plasticity underlying long-term potentiation and long-term depression (LTD) (Bredt and Nicoll, 2003). Although the density of GluR4-expressing AMPARs peaks during the first postnatal week but declines steadily to adult levels, GluR1/GluR2/GluR3-containing AMPARs increase gradually and stabilize at postnatal day 20 (Wentholt et al., 1996; Zhu et al., 2000). Synaptic activity drives membrane expression of GluR1-containing AMPARs, whereas GluR2/GluR3-containing AMPARs cycle constitutively on and off the synaptic membrane (Malinow et al., 2000; Shi et al., 2001) with their density regulated by synaptic activity (Liu and Cull-Candy, 2002). Accordingly, the subunit compositions and density of the membrane-associated AMPARs determine the basal synaptic strength and regulate synaptic plasticity (Turri-giano and Nelson, 2004). GluR2/GluR3 interact with synaptic

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Figure 1. Synaptic expression of GluR2 and GluR3 is markedly reduced in the frontal cortex (FCX) of prenatal cocaine-exposed rats. **a**, Western blot analysis of the GluR2 and GluR3 distribution in cytosol (Cy) and membrane (M) fractions of the synaptosomes prepared from FCX of P21 rats exposed to saline or cocaine during gestation. The blots were stripped and sequentially reprobed with anti-caspase-3 (1187 ± 107 and 1210 ± 99 optical intensity in saline and cocaine, respectively) and GRIP1 (2509 ± 132 and 2319 ± 146 optical intensity in saline and cocaine, respectively) to validate equal loading in cytosolic and membranous extracts. **b**, The GluR2 or GluR3 bands were quantified individually. Data are presented as means $\pm$ SEM of the optical intensity. $n = 4$ for each group. $*p < 0.01$ comparing the level of receptor in respective cytosol and membrane fractions. **c**, Immunohistochemical detection of GluR2 and GRIP1 in the frontal cortex of P21 prenatal saline- and cocaine-exposed rats. The representative sections show more GluR2 punctuates in membranes of saline-treated rats without discernible changes in GRIP1 levels. Scale bar, $50 \mu\text{m}$. $n = 4$ for each group.

scaffolding proteins: glutamate receptor-interacting protein (GRIP1/2) (Dong et al., 1997), AMPAR-binding protein (ABP) (Srivastava et al., 1998), and protein interacting with C kinase-1 (PICK1) (Xia et al., 1999). Although GRIP1/2 stabilizes GluR2/GluR3 at the synaptic membrane, PICK1 promotes their internalization into cytoplasm (Kim et al., 2001). Hence, the level of interaction between AMPARs and postsynaptic density-95/Disks

large/zona occludens-1 (PDZ)-containing proteins greatly impacts synaptic transmission, and any changes in these interactions may, therefore, alter synaptic activity.

In the present investigation, we test the hypothesis that reduced AMPAR density in synaptic membranes reduces AMPAR activation in prenatal cocaine-exposed brain. Our data identify a reduced GluR2/3-GRIP interaction as the primary mechanism through which prenatal cocaine exposure attenuates GluR2/GluR3 synaptic recruitment. This cocaine effect could delay brain maturation, resulting in cognitive impairments and vulnerability to neuropsychiatric disorders decades later.

Materials and Methods

Materials and chemicals. Soybean trypsin inhibitor, phenylmethylsulfonyl fluoride (PMSF), 2-mercaptoethanol, NaF, Na_2VO_4 , digitonin, protein phosphatase inhibitor I and II cocktails, recombinant γ protein kinase C (PKC), alkaline phosphatase, phorbol 12-myristate, 13-acetate (PMA), anti-phosphoserine (P3430), and anti-phosphothreonine (P3555) were purchased from Sigma. Leupeptin and aprotinin were from Peptide International. Recombinant Src, celestrine, and PP1 were from Calbiochem. Antibodies against GluR2 (SC-7610), GluR3 (SC-7613), phosphotyrosine (SC-508), PKC α (SC-208), PKC β (SC-209/210), PKC γ (SC-211), PKC δ (SC-937), PKC ϵ (SC-214), GRIP1 (SC-17641), GRIP2 (SC-15477), phosphotyrosine (SC-508), caspase-3 (SC-7272), *N*-ethylmaleimide-sensitive factor (NSF; SC-5828), PICK1 (SC-9541), and β -actin (SC-47778) were purchased from Santa Cruz Biotechnology. Antibodies against pThr^{638/641}PKC α / β II (#9375), pThr⁵¹⁴PKC γ (#9379), and pThr⁴¹⁰PKC/PKM ζ (#2060) were from Cell Signaling Technology. Seize-X immunoprecipitation kit, antigen elution buffer, and West pico chemiluminescent reagents were purchased from Pierce-Endogen. Bradford reagent, SDS-PAGE reagents, and prestained molecular weight markers were purchased from Bio-Rad. Cutoff filters (10 kDa) were obtained from Cole-Palmer. ABP (AB5569) and β -tubulin (MAB3408) were from Millipore Bioscience Research Reagents. Target buffer was purchased from Dako. Avidin-peroxidase-labeled biotin complex (ABC) was from Vector Laboratories. 3–3'-diaminobenzidine-4 HCl (DAB)/ H_2O_2 was from Biomed.

Animal treatment. Pathogen-free, 10-week-old male and female Sprague Dawley rats weighing ~ 200 – 215 g (Taconic) were housed individually in a 12 h light/dark cycle with *ad libitum* access to food and water. All animal procedures were in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the City College of New York Animal Care and Use Committee. A pair of 10-week-old male and female rats was placed in a cage overnight. In each experiment set, we used four mating pairs. The presence of a sperm-positive vaginal plug indicated gestational day (GD)

0. The pregnant female rats were housed individually without disturbance other than the daily injection with cocaine or saline. On GD 1, pregnant dams were assigned to receive daily subcutaneous injections from GD 1–21 of either cocaine HCl, 30 mg/kg in 0.9% saline or saline, 2 ml/kg. The animals were injected daily between 9:00–10:00 A.M. After each injection, these pregnant rats were observed for 1 h and behavioral abnormalities recorded. There were apparent increases in locomotor activity in cocaine-treated rats. To minimize skin lesions and tissue necrosis in the cocaine-injected rats, the injection sites were rotated over different sites on the back. There were no discernible differences in litter size (between 7–13 pups) and body weight of the pups at 21 d of age (48.9 ± 2.5 and 50.7 ± 2.8 g for cocaine and saline, respectively; $n = 40$ each) and gender distribution (23 males/17 females and 19 males/21 females for cocaine and saline groups, respectively). There was also no correlation between the magnitudes of tissue necrosis induced by subcutaneous cocaine injections and litter size or body weight of the pups. There were no obvious changes in rearing behaviors. Importantly, the dose of cocaine used in this study did not induce seizure or fatality.

In a separate experiment series, confirmed pregnant dams were assigned to receive daily intraperitoneal injections from GD 8–20 of either cocaine HCl, 30 mg/kg in 0.9% saline or saline, 2 ml/kg. The animals were also injected daily at 9:00–10:00 A.M.

The progenies were group-housed with their mother until being killed at 21 d of age postnatal day 21 (P21)]. Food and water were available *ad libitum*. They were subjected to the minimum handling associated with routine animal husbandry. Since we did not find gender differences in our previous studies conducted in rabbit and rats (H. Y. Wang et al., 1995; Jones et al., 2000; Yablonsky-Alter et al., 2005), both sexes from separate litters were used in these experiments. Pups were killed by rapid decapitation, the brain was removed immediately, and frontal cortices were dissected on ice.

Preparation of synaptosomes and fractionation. Synaptosomes (P2 fraction) were prepared from frontal cortices as described previously with few modifications (Wang et al., 1994, 1999). To further purify synaptosomal fractions, the synaptosome-enriched P2 fraction was washed twice in 5 ml of ice-cold Krebs–Ringer’s solution (25 mM HEPES, pH 7.4; 118 mM NaCl, 4.8 mM KCl, 25 mM NaHCO₃, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 10 mM glucose, 100 μ M ascorbic acid, 50 μ g/ml leupeptin, 10 μ g/ml aprotinin, 2 μ g/ml soybean trypsin inhibitor, 0.04 mM PMSF, 0.1 mM 2-mercaptoethanol, 10 mM NaF, 1 mM Na₂VO₄, and 0.5 μ l/ml protein phosphatase inhibitor I and II cocktails). To obtain cytosolic and membranous fractions of the synaptosomes, the washed synaptosomes were sonicated for 10 s on ice in 0.5 ml hypotonic homogenization solution (25 mM HEPES, pH 7.4; 120 mM NaCl, 4.8 mM KCl, 25 mM NaHCO₃, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 10 mM glucose, 100 μ M ascorbic acid, 50 μ g/ml leupeptin, 10 μ g/ml aprotinin, 2 μ g/ml soybean trypsin inhibitor, 0.04 mM PMSF, and 0.1 mM 2-mercaptoethanol, 10 mM NaF, 1 mM Na₂VO₄, and 0.5 μ l/ml protein phosphatase inhibitor I and II cocktails). Samples were centrifuged at $50,000 \times g$ for 30 min. The resultant supernatant was reserved as the cytosolic fraction, and the synaptic membrane pellet was resuspended in 0.5 ml of hypotonic solution. Protein concentrations of the synaptic membranes were determined us-

Figure 2. LTD in medial prefrontal cortical slices from prenatal cocaine- and saline-exposed rats. *a*, Field potential recording of electrically evoked responses before (baseline, single gray line) and at various times after (fine black lines) LFS in a representative slice from a prenatal cocaine-exposed rat. *b*, Matching experiment performed on a slice prepared from a prenatal saline-exposed rat, showing reduced response at the first negative wave and increased latency at the second wave. *c*, Amplitude of the responses in *a* relative to baseline (bln). Each bar represents mean amplitude $\pm$ SD of four traces measured at the first negative wave (arrowhead). *d*, Similar bar graph representing the relative amplitudes of post-LFS responses in *b* measured at the first negative wave (arrowhead). Note the less significant LTD displayed by the slice from the cocaine-exposed rat in *a* and *c*. ^o $p < 0.005$; * $p < 0.0005$; ** $p < 0.00005$ (Student's 2-tailed *t* test when compared with bln of each group). Between-group differences in time course of prenatal cocaine- versus saline-exposed brain were compared using two-factor ANOVA ($p < 0.01$). Calibration: 5 ms, 0.2 mV. $n = 4$ for each group. *e*, A typical field potential response evoked in Layer III of a rat mPFC slice by off-radius electrical pulse stimulation of Layer II showing negative waves labeled *a*, an AMPA receptor-mediated response component identified through its blockade by CNQX; and *b*, NMDA receptor-mediated components identified using APV, a selective NMDA blocker. The early part of the NMDA receptor response was sensitive to RO 25-6981, a blocker for the NR2B subunit-containing NMDA receptor, and it overlapped with the later part but not the peak of the AMPA receptor response. Calibration: 0.2 mV, 50 ms.

Table 1. Comparison of the number and percentage of tested animals showing LTD (>10% reduction from baseline) from prenatal saline- and prenatal cocaine-exposed rats

	Saline ($n = 15$)	Cocaine ($n = 16$)
Number showing LTD	11	7
% showing LTD	73%	43%
Decrease >20%	8	2

Shown in the last row are the number of animals exhibiting a >20% LTD. *n*, Number of slices recorded.

ing the Bradford method before solubilization by boiling for 5 min in $6\times$ SDS-PAGE sample preparation buffer.

Immunoaffinity purification of native GluR2, GluR3, GRIP1, and GRIP2. To isolate native GluR2, GluR3, GRIP1, and GRIP2, frontal cortices of P21 prenatal cocaine- or saline-exposed rats were homogenized in hypotonic homogenization solution as described above. The obtained homogenates were solubilized using 0.5% digitonin, 0.2% sodium cholate, 0.5% NP-40, and 0.2% SDS in the presence of cocktails of protease and protein phosphatase inhibitors for 20 min at 25°C followed by

Table 2. Mean amplitudes of the AMPA receptor component of the basal responses to a range of stimulus intensities in prenatal saline- and cocaine-exposed male rats

Stimulation intensity (a.u.)	Saline			Cocaine			
	Mean response amplitude (mV)	SEM		Mean response amplitude (mV)	SEM		
1	0.09	0.02	<i>n</i> = 21	0.13	0.03	<i>n</i> = 15	NS
1.5	0.62	0.13	<i>n</i> = 15	0.52	0.06	<i>n</i> = 8	NS
2	0.71	0.11	<i>n</i> = 21	0.69	0.08	<i>n</i> = 15	NS
3	0.93	0.13	<i>n</i> = 21	0.91	0.09	<i>n</i> = 15	NS

Student's two-tailed *t* test shows no significant (NS) difference between the two groups for any stimulus intensity in the range tested. a.u., Arbitrary units.

60 min at 4°C with end-over-end constant shaking. After centrifugation to remove insoluble debris, the obtained brain lysate was diluted fivefold, and GRIP1, GluR2, and GluR3 were individually purified using immunoaffinity columns (Seize-X immunoprecipitation kit) with covalently immobilized antibodies directed against GluR2, GluR3, GRIP1, and GRIP2, according to manufacturer's instructions. GluR2, GluR3, GRIP1, and GRIP2 were each eluted twice with 90 μ l antigen elution buffer. The resultant eluates were neutralized immediately with 20 μ l 1.5 M Tris, pH 8.8, and concentrated to 100 μ l by passing through a 10 kDa cutoff filter. Protein concentrations were determined using the Bradford method. The purity of each protein was validated by Western blotting. In each case, the purified protein yielded a single protein band with apparent molecular weight identical to that found using rat brain lysate.

In vitro determination of GluR2–GRIP1 interaction and immunoprecipitation. To control the GRIP1 phosphorylation state, native GRIP1 proteins (10 μ g) purified from frontal cortices of P21 prenatal saline- and cocaine-exposed rats were incubated with 100 μ g/ml alkaline phosphatase in Tris, pH 8.0, 130 mM NaCl, and protease inhibitors at 30°C for 20 min (total incubation volume 100 μ l). The phosphatase activity was terminated by 10 mM NaF/1 mM Na_3VO_4 , and specific PKC- and src-mediated phosphorylation was induced by incubation with 0.5 μ g/ml recombinant γ PKC, 20 μ g phosphotidylserine, and 100 nM PMA or 10 μ g/ml recombinant Src in the presence of 30 μ M ATP in Krebs–Ringer's at 30°C for 10 min (total incubation volume 125 μ l). The actions of PKC and Src were terminated by addition of 1 μ M celestirine and PP1, respectively. One-half of the GRIP1 solution (containing 5 μ g) was immediately solubilized by adding 6 $\times$ SDS-PAGE sample preparation buffer and boiled for 5 min for analysis of phosphoserine and phosphothreonine and phosphotyrosine levels by Western blotting. To determine the influence of GRIP1 phosphorylation state on the interaction between GRIP1 and GluR2, purified brain GluR2 (5 μ g) from gestational saline- and cocaine-exposed rats were individually added to 5 μ g of GRIP1 with different phosphorylation states and incubated in 100 μ g/ml brain phospholipids, 1% BSA-containing Krebs–Ringer's at 30°C for 30 min with constant end-over-end shaking. The GRIP1-associated GluR2 was isolated along with GRIP1 by 20 μ l immobilized anti-GRIP1-conjugated protein A-agarose beads and measured using Western blot with anti-GluR2. The obtained blots were screened for phosphoserine first (anti-phosphoserine), stripped, and reprobbed twice sequentially with anti-phosphotyrosine and anti-GRIP1. The signals were detected using a chemiluminescent method and visualized by exposure to x-ray film.

Western blotting. To determine cellular distribution or the interaction between GluR2 or GluR3 and GRIP1, cytosolic and membranous fractions of frontal cortices or anti-GRIP1 immunoprecipitates were boiled for 5 min in 100 μ l SDS-PAGE sample buffer (62.5 mM Tris-HCl, pH 6.8; 10% glycerol, 2% SDS; 5% 2-mercaptoethanol, 0.1% bromophenol blue) and then size fractionated on 7.5 or 10% SDS-PAGE based on the molecular mass of the protein. Proteins were electrophoretically transferred to nitrocellulose membrane, and Western blotting was performed with antibodies for GluR2, GluR3, phosphotyrosine, phosphoserine, phosphothreonine, PKC α , PKC β , PKC γ , PKC δ , PKC ϵ , pThr^{638/641}PKC α / β II, pThr⁵¹⁴PKC γ , and pThr⁴¹⁰PKC/PKM ζ . The blots were stripped and reprobbed with anti-GRIP1 or caspase-3 to assess the level of sample loading.

To determine the expression level of GluR2, GluR3, GRIP1, GRIP2, protein extracts of the synaptosome-enriched P2 fractions (50 μ g) were

size fractionated on 7.5% SDS-PAGE, and Western blotting was performed using antibodies against GluR2, GluR3, p-GluR2/3(Ser⁸⁸⁰/Ser⁸⁹¹), GRIP1, GRIP2, ABP, NSF, and PICK1. In some cases, the blots were stripped and reprobbed with anti- β -actin, β -tubulin, and GRIP1.

Immunoreactivity was detected by reacting with chemiluminescent reagents for exactly 5 min and visualized by immediately exposing to x-ray film for 10–30 s. Specific protein bands were quantified by densitometric scanning (GS-800 calibrated densitometer; Bio-Rad Laboratories).

Electrophysiological studies. To elucidate the impact of prenatal cocaine exposure on AMPAR function during brain maturation, electrophysiological recordings that focus on AMPAR function were performed in 400- μ m-thick medial prefrontal cortical slices (mPFC) from P21–P25 prenatal cocaine- or saline-exposed (and some treatment naive) rats. This procedure was performed as described previously (Otani et al., 1998) with modifications to determine AMPAR-mediated LTD.

Brain slice preparation. Rats were decapitated under anesthesia (60 mg/kg pentobarbital sodium) and the craniums quickly opened. Brains were removed and placed in ice-cold artificial CSF (ACSF) saturated with 5% CO_2 and 95% O_2 for 2 min, then blocked and sectioned into 400 μ m coronal slices using a Vibratome (TPI). Slices were incubated at room temperature ($\sim$ 25°C) in ACSF bubbled with the CO_2/O_2 gas mixture for 2 h before recording. ACSF contained 138 mM NaCl, 3.0 mM KCl, 1.2 mM NaH_2PO_4 , 20.25 mM NaHCO_3 , 2 mM CaCl_2 , 1.3 mM MgCl_2 , and 25 mM dextrose.

Electrophysiological recording. All brain slices were recorded in a submersion-type recording chamber (Warner Instruments) superfused (2 ml/min) with ACSF containing 80 μ M picrotoxin at room temperature (22–25°C). Evoked field potential in layer III of rat prefrontal cortical slices were recorded through a glass micropipette (with a 4–5 μ m tip) filled with 1 M Na acetate placed in the prelimbic area 300 μ m from the pia. To evoke baseline responses, single electrical pulses (0.1 ms, 0.1 Hz, at 2 $\times$ threshold strength) were delivered through a concentric bipolar electrode (Frederick Haer Corporation) placed on layer I/II 100 μ m lateral to the radial axis of the recording location to favor activation of axons from nearby columns (i.e., by across-column stimulation). Voltage responses were led to a d.c. amplifier (Axoclamp 2a) and digitized by a 16-bit analog-to-digital converter (Measurement Computing) operated through data-acquisition software (Snapmaster 3.5; Hem Corp). The responses were averaged over four frames to improve signal-to-noise ratio, then subsequently analyzed in real time.

LTD was elicited by a single train of low-frequency stimulation (LFS; 2.4 Hz of triplets at 500 Hz, for 10 min) delivered to the same location at the same strength as for baseline responses. After LFS, single-test responses were evoked for 40 min by the same way as for baseline responses and plotted against time to reveal any persistent depression from the baseline amplitude.

Immunohistochemistry. Immunohistochemical analyses were performed using antibodies directed against GluR2, GluR3, GRIP1, and GRIP2 and were performed on paraffin-embedded tissues as described previously (Nagele et al., 2002). Briefly, after removal of paraffin with xylene and rehydration through a graded series of decreasing concentrations of ethanol, protein antigenicity was enhanced by microwaving sections in target buffer for 2 min. After a 30 min incubation in 0.3% H_2O_2 , sections were treated for 30 min in normal blocking serum and then incubated with primary antibodies at appropriate dilutions for 1 h at room temperature. After a thorough rinse in PBS, a secondary biotin-labeled antibody was applied for 30 min. Immunoreactions were treated with the ABC and visualized by treatment of sections with DAB/ H_2O_2 . Sections were lightly counterstained with hematoxylin, dehydrated through a graded series of increasing concentrations of ethanols, cleared in xylene, and mounted in Permount. Controls consisted of comparable sections treated with nonimmune serum, preabsorbed antibody, or omission of the primary antibody. Specimens were examined and pho-

tographed with a Nikon FXA microscope, and digital images were recorded using a Nikon DXM1200F digital camera and processed using Image Pro Plus (Phase 3 Imaging) imaging software.

Data analysis and statistical evaluation. Statistical differences between cocaine and saline groups were assessed using the two-tailed Student's *t* test. Differences between *in vitro* dose-response relations were analyzed by ANOVA followed by Newman–Keuls multiple comparisons.

Results

Prenatal cocaine reduces the synaptic targeting of GluR2 and GluR3 subunits

The effect of prenatal cocaine exposure during the entire gestation on the distribution of GluR2- and GluR3-containing AMPARs was determined in synaptosomes prepared from the frontal cortices of 21-d-old (P21) rats prenatally exposed to cocaine or saline (control). We measured the relative levels of GluR2 and GluR3 in the cytosol and synaptic membranes of these synaptosomes. Although the majority of GluR2 ($89.7 \pm 6.2\%$) and GluR3 ($94.1 \pm 5.1\%$) are located in synaptic membrane of saline-treated brains, prenatal cocaine exposure dramatically reduced synaptic membrane-associated GluR2 and GluR3 levels, concurrent with corresponding increases in the abundance of cytosolic GluR2 ($62.3 \pm 5.1\%$ of the overall GluR2 expression levels in synaptosomes) and GluR3 ($58.6 \pm 4.5\%$ of the overall GluR3 expression levels in synaptosomes) (Fig. 1*a,b*). The higher level of membrane-localized GluR2 in prenatal saline-treated brains was consistent with the immunohistochemical results that show more GluR2-immunoreactive puncta on membranes (Fig. 1*c*). Similarly, significant retention of GluR2 and GluR3 in the cytosol was also observed in synaptosomes of frontal cortices from 21-d-old rats exposed to cocaine during gestation days 8–20 (supplemental Fig. 1, available at www.jneurosci.org as supplemental material).

Since GluR1-containing AMPARs and the constitutive cycling pool of GluR2- and GluR3-containing AMPARs play crucial roles in the maintenance of basal synaptic transmission and LTD expression, respectively (Chung et al., 2000; Kim et al., 2001), we tested whether the AMPAR-mediated basal field potential response and LTD are altered by prenatal cocaine exposure. The magnitude of AMPAR-mediated LTD in the presence of GABA_AR blockade by picrotoxin was compared in the mPFC from P21 to P25 prenatal saline- and cocaine-exposed rats. The experiment illustrated in Figure 2*a–d* compared the amplitude of the earliest negative

Figure 3. Prenatal cocaine exposure did not alter the expression levels of GRIP1 or GRIP2, GluR2 or GluR3 or pS⁸⁸⁰-GluR2 or pS⁸⁹¹-GluR3 (*a, c, e*). The expression levels of GRIP1, GRIP2, GluR2, GluR3, pS⁸⁸⁰-GluR2 and pS⁸⁹¹-GluR3 in postmitochondrial synaptosome-enriched fraction prepared from frontal cortices of P21 rats exposed to saline or cocaine *in utero* were compared by Western blotting either directly (*a, c*) or after immunoprecipitation with anti-GluR2 or anti-GluR3 (*e*). The blots were stripped and sequentially reprobbed with anti-β-tubulin (*a*), anti-β-actin (*c*), or anti-GluR2 and anti-GluR3 (*e*) to validate equal loading and/or immunoprecipitation efficiency. Protein bands were quantified and normalized with respect to signals of loading controls (*b, d, f*). Between-group differences were not statistically significant (*b, d, f*). Data are presented as means $\pm$ SEM. *n* = 4 for each group.

wave (arrowhead) in the evoked field potential before and after LFS. In separate tests, this early wave was blocked by an AMPAR-selective antagonist CNQX (Fig. 2*e*). Figure 2 showed that LFS generated a discernibly milder LTD of the AMPAR response in

Figure 4. Prenatal cocaine exposure did not alter expression levels of ABP, NSF, and PICK1. Expression levels of ABP, NSF, and PICK1 in postmitochondrial synaptosome-enriched fraction prepared from frontal cortices of P21 rats exposed to saline or cocaine *in utero* were compared by Western blotting. The blots were stripped and reprobed with anti-β-actin to validate equal loading. **a**, The blot is a representative of four individual rats from each treatment group. Densitometric quantification of blots shown in **b**; $n = 4$. Data are means $\pm$ SEM of the ratios of optical intensity for the indicated protein to loading control, β-actin (**b**). There were no statistical differences between prenatal saline- and cocaine-exposed rats in these measures.

Figure 5. Prenatal cocaine exposure did not alter the association levels of GluR2 or GluR3 with PICK1. **a**, Coupling between GluR2 or GluR3 and synaptic scaffolding protein, PICK1, in the postmitochondrial synaptosome-enriched fraction prepared from frontal cortices of P21 rats exposed to saline or cocaine *in utero* were compared by Western blotting after immunoprecipitation with anti-PICK1. Blots were stripped and reprobed with anti-PICK1 to validate equal loading and immunoprecipitation efficiency. The blot is a representative of four individual rats from each treatment group (**a**). **b**, Densitometric quantification of four blots identical to that shown in **a** expressed as means $\pm$ SEM of the ratios of optical intensities for GluR2 or GluR3 to PICK1. $n = 4$. There were no statistical differences between prenatal saline- and cocaine-exposed rats in these measures.

the slice from a prenatal cocaine-exposed rat (Fig. 2*a,c*) than that from a matched prenatal saline-exposed control rat (Fig. 2*b,d*). Among all the slices tested, 43% (7 of 16) from prenatal cocaine-exposed rats showed significant depression, whereas 73% (11 of 15) of slices from prenatal saline-exposed rats showed significant depression. In addition, four times more slices in the saline control group showed $>20\%$ depression (Table 1). This reduction in LTD occurred without any discernible change in basal synaptic transmission tested at a large range of stimulus strengths (Table 2, $\sim 1\text{--}4 \times$ threshold current). Absence of effect of prenatal cocaine exposure on baseline electrically evoked field potential responses was also confirmed when the magnitude of evoked responses measured as amplitude of the first negativity and as initial slope of the evoked responses both plotted against stimulus current strengths in mPFC slices from prenatal cocaine- and saline-exposed P21 rats (supplemental Fig. 2, available at www.jneurosci.org as supplemental material).

Similar deficits in LTD also found in mPFC of P21 rats exposed to cocaine during gestation days 8–20 (data not shown).

In a systematic approach to assessing the potential mechanism underlying prenatal cocaine-induced impairment of AMPAR-mediated LTD, we first considered changes in the overall expression of AMPAR GluR2 and GluR3 subunits and their synaptic scaffolding partner GRIP1/2. Because the phosphorylation of GluR2/3 at pSer⁸⁸⁰-GluR2 and pSer⁸⁹¹-GluR3 by PKC promotes their interaction with PICK1 and subsequent internalization (Chung et al., 2000), we explored the possibility that an altered phosphorylation state of these subunits may have resulted in internalization of GluR2/3 in the prenatal cocaine-exposed brain. However, Western blot data showed that prenatal cocaine exposure did not alter the overall expression of GRIP1 and GRIP2 (Fig. 3*a*), GluR2 and GluR3 (Fig. 3*c*), pSer⁸⁸⁰-GluR2 and pSer⁸⁹¹-GluR3 (Fig. 3*e*). Prenatal cocaine exposure also did not alter the synaptic levels of scaffolding proteins PICK1, ABP, or the non-PDZ domain containing the AMPAR-interacting protein, NSF (Fig. 4). Since an increased interaction between PICK1 and GluR2/3 could also reduce AMPAR levels on synaptic membrane through enhancing internalization of these AMPAR subunits, we compared the levels of PICK1-associated GluR2 and GluR3 in prenatal saline- and cocaine-exposed brains by Western blotting with anti-GluR2 and GluR3 in anti-PICK1 immunoprecipitates. Our data presented in Figure 5 indicate that prenatal cocaine exposure did not affect PICK1–GluR2/3 interaction as similar GluR2 and GluR3 levels were found in PICK1 immunoprecipitates from prenatal cocaine- and saline-exposed groups. Together, these data

suggest that the apparent retention of GluR2/3 in the cytosol of prenatal cocaine-exposed brains was not the result of altered overall expression of GluR2/3 and synaptic scaffolding molecules, phosphorylated GluR2/3, or their interaction with PICK1.

Prenatal cocaine exposure reduces the interaction between GluR2/3-GRIP

Because GluR2/3 localize in synaptic membranes by interacting with GRIP1/2, we hypothesize that a reduced GluR2/3 interaction with GRIP1/2 in prenatal cocaine-exposed brains may be responsible for the diminished GluR2/3 in synaptic membranes. To directly test this possibility, we first individually isolated native GluR2, GluR3, and GRIP1 from frontal cortices of P21 rats prenatally exposed to saline or cocaine using immunoaffinity columns with immobilized antibodies to each of these proteins.

Figure 6. Reduced association between GluR2/GluR3 and GRIP1 in the frontal cortex of prenatal cocaine exposed rats. **a**, Native GRIP1, GluR2, and GluR3 were individually purified from frontal cortices of P21 rats exposed to saline (S) or cocaine (C) during gestation by immunoaffinity columns with immobilized antibodies in the presence of protease and protein phosphatase inhibitors. The interaction between GluR2 or GluR3, and GRIP1 was assessed by measuring levels of GluR2 or GluR3 in purified GluR2- or GluR3-GRIP1 complexes using Western blotting. **b**, Densitometric quantification of GluR2 or GluR3 bands. Data are presented as means $\pm$ SEM of optical intensities. $n = 4$ for each group. $*p < 0.01$ comparing the level of GluR2 or GluR3 in the indicated condition with the group that GRIP1 and receptor are both isolated from prenatal saline-treated rats by Newman-Keuls multiple comparisons that followed one-way ANOVA. No between-group differences were found in GRIP1 level.

To identify the mechanism underlying the prenatal cocaine-induced reduction in synaptic membrane expression of GluR2/3, purified GluR2, GluR3, and GRIP1 in frontal cortices from rats prenatally exposed to saline or cocaine were combined separately *in vitro* using a cross-over design. After isolation of GRIP1-associated GluR2 and GluR3 by coimmunoprecipitation with anti-GRIP1 antibodies, the levels of GluR2 or GluR3 were determined by Western blotting with anti-GluR2 or anti-GluR3 antibodies. The data show that GluR2 and GluR3 isolated from rat brains prenatally exposed either to saline or cocaine bind equally well to GRIP1 from saline-exposed tissues (Fig. 6*a,b*). In contrast, the association of GluR2 or GluR3 derived from rats in either treatment group with GRIP1 from prenatal cocaine-exposed rats was markedly reduced (Fig. 6*a,b*). These data together indicate that the reduced interaction between GRIP and GluR2/3 in frontal cortices of rats prenatally exposed to cocaine is caused by a persistent modification of the synaptic scaffolding protein GRIP.

PKC- and Src-mediated GRIP1 phosphorylation disrupts

GRIP1–GluR2 coupling in the prenatal cocaine-exposed brain
Among various potential mechanisms that may change the capability of GRIP to interact with GluR2/3, we considered an altered phosphorylation state of GRIP as the most plausible. GRIP har-

Figure 7. Enhanced serine and tyrosine phosphorylation of GRIP1 and GRIP2 in frontal cortices of prenatal cocaine-exposed rats. **a**, Levels of Serine (Ser), Threonine (Thr), and tyrosine (Tyr) phosphorylation on purified native GRIP1 or GRIP2 from prenatal saline- and cocaine-exposed P21 rats were determined by Western blot using specific antibodies directed against each phosphoepitope. **b**, Densitometric quantification of blots. Data are represented as means $\pm$ SEM of the optical intensity. $n = 4$ for each group. $*p < 0.01$ comparing the levels of phosphorylated GRIP1 and GRIP2 isolated from prenatal saline-treated rats by two-tailed Student's *t* test. No between-group differences in the levels of GRIP1 immunoprecipitates were detected.

bors putative phosphorylation sites for PKC and other kinases. To investigate how an altered phosphorylation state of GRIP may influence its capacity to anchor with GluR2/3, we compared the phosphorylation states of GRIP using purified native GRIP from frontal cortices of P21 rats prenatally exposed to saline or cocaine. Using Western blotting with phosphoepitope-specific antibodies, we found that both GRIP1 and GRIP2 derived from prenatal cocaine-exposed rats showed at least a twofold increase in serine (pSer) and tyrosine (pTyr) residues without signs of threonine (pThr) phosphorylation (Fig. 7*a,b*). Similarly, exposure to cocaine during gestation days 8–20 also increased serine and tyrosine phosphorylation on GRIP1 (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). To further assess whether an increased pSer- and pTyr-GRIP is responsible for the reduced GluR2/3–GRIP interaction in prenatal cocaine-exposed brain, we manipulated *in vitro* the phosphorylation state of the purified GRIP1 from prenatal cocaine- or saline-exposed

Figure 8. PKC- and Src-mediated phosphorylation of GRIP1 reduces GluR2–GRIP1 coupling in prenatal cocaine-exposed P21 rats. *a*, Immunoaffinity-purified native GRIP1 from frontal cortices of either prenatal saline (S)- or cocaine (C)-exposed rats was treated with vehicle or 100 μ g/ml alkaline phosphatase. After completion of dephosphorylation by addition of inhibitors, phosphate-free GRIP1 was phosphorylated by recombinant PKC or Src in the presence of ATP. The reaction was terminated by specific PKC or Src inhibitors, and GRIP1 was analyzed for phosphoserine and phosphotyrosine by Western blotting. The interaction between GluR2 and GRIP1 with different phosphorylation states was assessed *in vitro* by incubation of purified GluR2 derived from either prenatal saline- or cocaine-exposed rats with purified, phosphorylated GRIP1. GluR2–GRIP1 complexes were isolated along with GRIP1 by immunoprecipitation, and levels of GluR2 associated with GRIP1 were determined by Western blotting. *b*, Densitometric quantification of the top blot shown in *a*. $n = 4$ for each group. Data are means $\pm$ SEM of the optical intensity. The statistical significance was evaluated by Newman–Keuls multiple comparisons that followed one-way ANOVA. * $p < 0.01$ comparing to native GRIP1 from saline-treated group. [#] $p < 0.01$ comparing with dephosphorylated GRIP1 from both saline- and cocaine-treated groups. ⁺ $p < 0.01$ comparing with native GRIP1 from cocaine-treated group.

rats and determined the resulting level of GluR2–GRIP1 association. Dephosphorylation of GRIP1 by alkaline phosphatase treatment restored the capacity of GRIP1 from prenatal cocaine-exposed brains to interact with GluR2 (Fig. 8*a,b*). Incubation of completely de-phosphorylated GRIP1 from either saline- or cocaine-exposed tissues with activated recombinant PKC or Src significantly reduced the level of GluR2–GRIP1 interaction (Fig. 8*a,b*). Last, since PKC activation has been shown to activate Src (Brandt et al., 2003) and since increased PKC translocation and activation has been demonstrated in rabbit brain after prenatal cocaine exposure (Wang et al., 1993), we assessed the activation of PKC. We found that levels of synaptic membrane-associated typical and atypical PKC isoforms in frontal cortices of prenatal cocaine-exposed rats were much higher than in frontal cortices of saline controls (Fig. 9*a,b*). In addition, substantially higher asso-

ciation between GRIP1 and activated PKC- γ , PKC- α , and PKC- ζ as well as PKM ζ were noted in frontal cortices from P21 prenatal cocaine-exposed rats (Fig. 9*c,d*). Collectively, these data demonstrate that prenatal cocaine exposure promotes PKC translocation and activation leading to increase in GRIP–PKC association and eventual PKC- and Src-mediated phosphorylation of GRIP on serine and tyrosine residues. This PKC- and Src-mediated GRIP phosphorylation, in turn, reduces the capacity of GRIP to interact with GluR2/3.

Discussion

Studies in both humans and animal models indicate that prenatal cocaine exposure can cause impairment in attention, motor, and language skills, as well as associative and discrimination learning, all of which involve excitatory synapses (Mayes et al., 1995; Romano and Harvey, 1996; Delaney-Black et al., 1996; Bandstra et al., 2002). These findings suggest that exposure to cocaine during early development can modify synaptic plasticity at the excitatory synapses resulting in enduring changes in brain function. In support of this possibility, our data indicate that *in utero* cocaine exposure attenuates AMPAR-mediated LTD without affecting basal transmission. The reduced AMPAR–LTD in prenatal cocaine-exposed brain is accompanied by reduced AMPARs in synaptic membranes (with apparent retention of GluR2/3 in cytosol), resulting from disrupted GluR2/3–GRIP interaction. This finding concurs with previous demonstrations that disruption of AMPAR constitutive cycling occludes LTD in hippocampal slices (Lüthi et al., 1999; Lüscher et al., 1999). Also resonating with our work showing that prenatal cocaine appears to attenuate AMPAR-mediated LTD without affecting basal transmission is the finding that prevention of GluR2 from binding to PDZ domain in anterior cingulate cortical slices blocks LTD without affecting basal synaptic transmission (Toyoda et al., 2007). Alternatively, the reduced LTD with decreased AMPARs in synaptic membranes observed here may be caused by defective metabotropic glutamate receptor (mGluR)1 signaling in prenatal cocaine-exposed brains (data not shown), since selective blockade of hippocampal mGluR1 reduces LTD expression and decreases AMPAR surface expression (Volk et al., 2006). The reduced AMPAR–LTD with normal basal transmission in prenatal cocaine-exposed brain may suggest that while the lower AMPARs in synaptic membranes are able to sustain basal synaptic activity, the reduced synaptic expression and/or a defective downstream mechanism underlying LTD lead to failure in LTD induction. The attenuated LTD in prenatal cocaine-exposed brains, however, is not likely the result of cocaine withdrawal, since 21 d of abstinence increases the surface to intracellular ratio of AMPARs (Boudreau and Wolf, 2005; Boudreau et al., 2007; Kourrich et al., 2007). With increasing evidence implicating LTD, especially in the NAc, as a pivotal mediator of drug-induced neuronal plasticity associated with drug addiction (for review, see Brebner et al., 2006), the impaired AMPAR-mediated LTD found in the prenatal cocaine-exposed brain may contribute to cognitive deficits in subjects exposed to cocaine *in utero*.

The involvement of AMPARs in reward processing has been well documented in adult animals exposed to cocaine (Sutton et al., 2003; Suto et al., 2004; Boudreau and Wolf, 2005; Mead et al., 2007; Conrad et al., 2008; Torregrossa et al., 2008; Famous et al., 2008). An increased AMPARs surface expression in NAc noted in cocaine-sensitized rats 21 d after last injection suggest that the elevated AMPARs in synapses enable drug-seeking responses (Boudreau and Wolf, 2005). In contrast, pharmacological inhibition of AMPARs (Kaddis et al., 1995; Bäckström and Hyttä,

Figure 9. Increased activated PKC- α , PKC- γ , and PKC/PKM- ζ association with GRIP1 in neuronal membranes from the frontal cortex of prenatal cocaine-exposed rats. **a**, Western blot analysis of the PKC isozyme distribution in the cytosol (Cy) and membrane (M) fractions of synaptosomes prepared from frontal cortices of P21 rats exposed to saline or cocaine during gestation. Blots were stripped and sequentially reprobed with anti-caspase-3 (996 ± 121 and 874 ± 119 optical intensity in saline and cocaine, respectively) and GRIP1 (2349 ± 165 and 2238 ± 151 optical intensity in saline and cocaine, respectively) to validate equal loading in cytosol and membrane extracts. **b**, Densitometric quantification of blots. **c**, Western blot analysis of the overall and activated PKC/M isozymes associated with GRIP1 in neuronal membranes from prenatal saline- and cocaine-exposed P21 rats. **d**, Densitometric quantification of blots. $n = 4$ for each group. Data are presented as means $\pm$ SEM of the optical intensities. * $p < 0.01$ comparing the level of the indicated PKC isozyme in respective cytosol and membrane fractions assessed by the two-tailed Student's t test.

2003; Harris and Aston-Jones, 2003; Choi et al., 2005; Torregrossa et al., 2008) or GluR1 and GluR2 knockdown (Dong et al., 2004; Mead et al., 2005) attenuates reward by brain stimulation, cocaine, or food. Additionally, studies have shown that rabbits exposed to cocaine *in utero* are tolerant to cocaine-induced motor sensitization, considered a behavioral correlate of addic-

tive processes (Stanwood and Levitt, 2003; Thompson et al., 2005). Our data showing reduced synaptic surface GluR2, GluR3 (Fig. 3), and GluR1 (data not shown) in prenatal cocaine-exposed brains concurs with these findings, implicating reduced AMPAR synaptic membrane localization and LTD expression in the impaired reward processing and tolerance to addictive drugs that

occur in the offspring of maternal cocaine users. The impairments in food reward in GluR2-deficient mice may also imply that subjects exposed to cocaine during gestation may be at greater risk of depression (Mead et al., 2005). In agreement with this depression-prone hypothesis, prenatal cocaine-exposed rats are significantly more immobile in the forced-swim test at 2 and 4 months of age (Overstreet et al., 2000).

The reduced GluR2/3 in synaptic membranes observed in prenatal cocaine-exposed brain is caused by an attenuated interaction between GluR2/3 and GRIP, the AMPAR synaptic anchoring protein, and not by altered expression of GluR2, GluR3, GRIP1, or GRIP2 or by increased internalization resulting from enhanced pSerGluR2/3–PICK interaction. Incidentally, we have observed fewer GluR1s in the synaptic membranes of prenatal cocaine-exposed brains (data not shown). Hence, a profound reduction in the synaptic membrane localization of AMPARs observed in prenatal cocaine-exposed brain coupled with an attenuated AMPAR-mediated LTD likely contributes to the cognitive changes, including impaired reward processing, exhibited by subjects exposed to cocaine during gestation.

Our data indicate that the reduced GluR2/3–GRIP interaction in prenatal cocaine-exposed brains is the result of a sustained PKC- and Src-mediated phosphorylation of GRIP, the synaptic anchoring protein for GluR2- and GluR3-containing AMPARs. This observation is reminiscent of a previous report that an increased phosphorylation of D_{1A} dopamine receptors in prenatal cocaine-exposed rabbits results in uncoupling of D_{1A} dopamine receptors from their signal transducer, G_s and G_o proteins (Zhen et al., 2001). A sustained PKC activation indicated by an overwhelming presence of synaptic membrane-associated multiple PKC isoforms and a markedly reduced phorbol ester-induced PKC translocation have also been observed in adult rabbit brains exposed to cocaine *in utero* (Wang et al., 1993). Similarly, we show here that prenatal cocaine treatment throughout gestation promotes cytosol-to-membrane translocation and activation of PKC together with markedly increased association of GRIP with activated PKC- α , PKC- γ and PKC- ζ as well as PKM ζ . Since PKC is known to activate Src (Brandt et al., 2003), our data supports the notion that prenatal cocaine exposure hyper-activates PKC, resulting in persistent PKC- and Src-mediated phosphorylation of GRIP, which in turn prevents GRIP from interacting with GluR2-, GluR3-containing AMPARs. In keeping with our theory that PKC hyperactivation profoundly affects cognitive processing in prenatal cocaine-exposed individuals, excessive PKC activation was found to markedly impair prefrontal cortex-mediated cognitive functions and increase distractibility (Birnbaum et al., 2004). Future experiments are needed to determine whether the reduced synaptic targeting of GluR2- and GluR3-containing AMPARs that we observed in brains from P21 prenatal cocaine-exposed rats is persistent or simply a transient shift in synaptic plasticity during early development. Nevertheless, previous studies conducted by us and others in rabbit indicate that such synaptic plasticity changes are long lasting, well into adulthood (Wang et al., 1993; Romano and Harvey, 1996; Stanwood and Levitt, 2003).

In addition to anchoring AMPARs to synaptic membranes, GRIP also interacts with other signaling molecules including GRIP-associated protein 1 (GRASP-1) (Ye et al., 2000), liprin- α (Wyszynski et al., 2002), ephrin B receptors (Hoogenraad et al., 2005), and matrix metalloproteinase 5 (Monea et al., 2006). Although the precise mechanism through which GRIP-interacting-signaling molecules contribute to the reduced GluR2/3 synaptic membrane localization in prenatal cocaine-exposed brain re-

mains ambiguous, a previous demonstration that overexpression of GRASP-1 in cultured hippocampal neurons reduces AMPAR synaptic targeting suggests that an overly active or abundant GRASP-1 may be a likely mediator (Ye et al., 2000).

Prenatal cocaine exposure has also been shown to affect numerous neurotransmitter systems including dopamine D₁ receptor–G_s/olf coupling (H. Y. Wang et al., 1995; Friedman et al., 1996; Jones et al., 2000; Zhen et al., 2001), GABAergic neurons (X. H. Wang et al., 1995), and noradrenergic system (Booze et al., 2006). Defective D₁ receptor signaling may itself contribute to the reduced AMPAR synaptic membrane localization, since D₁ receptor stimulation recruits AMPARs to synaptic membranes (Mangiacavalli and Wolf, 2004). Despite these numerous changes reported to occur in prenatal cocaine-exposed brains, we propose that hyper-activation of PKC resulting in sustained phosphorylation of GRIPs and reduced GluR2/3 synaptic membrane targeting elucidated here may be a prominent cascade of events underlying prenatal cocaine-induced cognitive impairment. Moreover, our data suggest that blocking excessive PKC translocation, a primary PKC activation mechanism, may be effective in preventing prenatal cocaine from promoting protracted deficits in AMPAR-regulated neurotransmission. Although mood stabilizers such as lithium and valproate may have potential therapeutic value as they block PKC translocation without interfering with the enzymatic activity (Hahn et al., 2005), lithium has also been shown to reduce AMPAR GluR1 and GluR2 synaptic expression (Du et al., 2003; Gray et al., 2003). Hence, among currently marketed drugs, treatments combining mood stabilizers that block PKC activation (Hahn et al., 2005) and antidepressants shown to boost AMPAR levels in synapses may have utility in maintaining proper AMPAR-regulated synaptic activities after prenatal cocaine exposure.

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