Cellular/Molecular

Cocaine Enhances NMDA Receptor-Mediated Currents in Ventral Tegmental Area Cells via Dopamine D_5 Receptor-Dependent Redistribution of NMDA Receptors

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Cocaine-induced plasticity of glutamatergic synaptic transmission in the ventral tegmental area (VTA) plays an important role in brain adaptations that promote addictive behaviors. However, the mechanisms responsible for triggering these synaptic changes are unknown. Here, we examined the effects of acute cocaine application on glutamatergic synaptic transmission in rat midbrain slices. Cocaine caused a delayed increase in NMDA receptor (NMDAR)-mediated synaptic currents in putative VTA dopamine (DA) cells. This effect was mimicked by a specific DA reuptake inhibitor and by a DA D_1/D_5 receptor agonist. The effect of cocaine was blocked by a DA D_1/D_5 receptor antagonist as well as by inhibitors of the cAMP/cAMP-dependent protein kinase A (PKA) pathway. Furthermore, biochemical analysis showed an increase in the immunoreactivity of the NMDAR subunits NR1 and NR2B and their redistribution to the synaptic membranes in VTA neurons. Accordingly, NMDAR-mediated EPSC decay time kinetics were significantly slower after cocaine, suggesting an increased number of NR2B-containing NMDARs. Finally, pharmacological analysis indicates that NR2B subunits might be incorporated in triheteromeric NR1/NR2A/NR2B complexes rather than in "pure" NR1/NR2B NMDA receptors. Together, our data suggest that acute cocaine increases NMDAR function in the VTA via activation of the cAMP/PKA pathway mediated by a DA D_5-like receptor, leading to the insertion of NR2B-containing NMDARs in the membrane. These results provide a potential mechanism by which acute cocaine promotes synaptic plasticity of VTA neurons, which could ultimately lead to the development of addictive behaviors.

Key words: **synaptic transmission; receptor trafficking; addiction; NMDAR; dopamine; cocaine**

Introduction

Behavioral sensitization is a process whereby repeated exposure to drugs of abuse such as the psychostimulants cocaine and amphetamine leads to a successively greater behavioral response to the drug (Post and Rose, 1976). The neurobiological changes leading to sensitization have been suggested to reflect drug-induced neuroplasticity in circuits that increase the incentive value of drugs (Robinson and Berridge, 1993). Thus, locomotor sensitization in rodents serves as a useful model to study changes in the brain responsible for the development of addiction. Adaptations related to sensitization were found in brain regions involved in psychomotor activation and reward, including the mesolimbic dopamine (DA) system (Kalivas and Stewart, 1991). A central role for NMDA receptor (NMDAR) in psychostimulant sensitization was first suggested by Karler et al. (1989), who found that systemic injections of the NMDAR antagonist MK-801 [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine maleate] blocked the development of sensitization to cocaine and amphetamine. It was subsequently found that NMDARs in the ventral tegmental area (VTA) are critical for the development of sensitization to cocaine (Kalivas and Alesdatter, 1993). A large body of evidence supported the hypothesis that the development of locomotor sensitization involved an enhancement of glutamatergic synaptic transmission in DA cells of the VTA (for review, see Wolf, 1998; Nestler, 2001; Kauer, 2003; Jones and Bonci, 2005). We observed that 1 d after a single *in vivo* injection, cocaine produces a long-term potentiation (LTP)-like enhancement of AMPA receptor (AMPAR)-mediated synaptic transmission in midbrain DA neurons. Although no changes of NMDAR-mediated currents were observed at this time, cocaine-induced LTP in the VTA was NMDAR dependent, because coinjection of the NMDAR antagonist with cocaine blocked both the development of the cocaine-induced LTP and context-dependent behavioral sensitization (Ungless et al., 2001). This suggests that at some point between the time of the *in vivo* cocaine injection and

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the electrophysiological recordings performed after at least 24 h, NMDAR activity in the VTA is required for the cocaine-induced LTP to be expressed. The finding that repeated injections of cocaine directly into the VTA are sufficient to produce locomotor sensitization suggests that some of the effects of cocaine on glutamatergic transmission, necessary to develop behavioral sensitization, occur locally within this structure (Cornish and Kalivas, 2001). Therefore, to test this possibility, we examined the effects of a brief acute cocaine application on AMPAR- and NMDAR-mediated responses in the VTA.

Materials and Methods

Reagents. The polyclonal anti-NR2A, anti-NR2B, anti-NR2C, anti-NR2D, and anti-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The polyclonal anti-pY1472 and anti-glutamate receptor 1 (GluR1) antibodies were purchased from Millipore (Billerica, MA). The monoclonal anti-NR1 antibody was purchased from Zymed Laboratories (South San Francisco, CA). The monoclonal anti-postsynaptic density-95 (PSD-95) antibodies were purchased from Millipore. The monoclonal anti-synaptophysin antibodies, picrotoxin, lidocaine, cocaine, *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH23390), (±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide (SKF81297), 1-(2-[bis-(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine (GBR12909), ifenprodil, *R*-(*R*,*S*)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidine propanol (Ro25-6981), and protein kinase inhibitor (PKI) were all purchased from Sigma (St. Louis, MO). (*R*)-[(*S*)-1-(4-bromo-phenyl)]-(2,3-dioxo-1,2,3,4-tetrahydro-quinoxalin-5-yl)-methyl]phosphonic acid (NVP-AAM077) was a generous gift from Dr. Y. P. Auberson (Novartis Pharmaceutical, Basel, Switzerland). Rp-cAMPs was purchased from Biolog (Göttingen, Germany). Anisomycin was purchased from Tocris Bioscience (Bristol, UK).

Electrophysiology. Sprague Dawley rats (21–29 d of age) were anesthetized and killed. The brain was rapidly dissected, and a tissue block containing the midbrain was used to cut horizontal slices on a vibratome (Leica, Nussloch, Germany) in ice-cold artificial CSF (aCSF) containing the following (in mM): 126 NaCl, 1.6 KCl, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 18 $NaHCO_3$, and 11 glucose (saturated with 95% O_2 and 5% CO_2). Slices were allowed to recover for at least 1 h in aCSF and were then transferred to a chamber with continuous flow (2.5 ml/min) of aCSF that contained 100 μM picrotoxin to block $GABA_A$ receptor-mediated synaptic currents. Cells were visualized using an upright microscope with infrared illumination. Whole-cell voltage-clamp recordings were performed using an Axopatch 1D amplifier (Molecular Devices, Foster City, CA). Electrodes pulled from glass capillaries (2–4 MΩ resistance) were filled with a solution containing the following (in mM): 117 $CsCH_3SO_3$, 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 $N(CH_2CH_3)_4Cl$, 2.5 Mg-ATP, and 0.25 Mg-GTP, pH 7.2–7.4. Putative DA cells were identified by the presence of a large hyperpolarization-activated potassium current, I_h (Lacey et al., 1989). I_h is present in ~70% of VTA DA neurons, and there is general agreement that GABAergic neurons do not have I_h (Cameron et al., 1997; Jones and Kauer, 1999; Neuhoff et al., 2002; Margolis et al., 2003). Although the presence of an I_h current does not unequivocally identify DA cells, it is likely that the contribution of I_h-positive tyrosine hydroxylase-negative cells to the observed effects is very small. A bipolar stimulating electrode was placed rostrally at a distance of 100–300 μm from the recording electrode with a mediolateral position optimal for evoking synaptic activity. Afferents were stimulated at 0.1 Hz, and the evoked EPSCs were filtered at 2 kHz, digitized at 5–10 kHz, and recorded using IgorPro software (Wavemetrics, Lake Oswego, OR). The paired-pulse protocol consisted of two paired stimuli delivered 50 ms apart. Unless indicated differently, all cells were recorded at a holding potential of +40 mV.

Data were averaged in 2.5 min bins, normalized to baseline, which is defined as the average EPSC amplitude of 10 min before drug application, and presented as average across cells ± SEM. The magnitude of the enhancement of the NMDAR-mediated component of the EPSCs (NMDAR EPSCs) was evaluated statistically, using paired Student's *t* test comparing the mean normalized EPSC amplitude during the final 30 sweeps of the baseline period with the mean EPSC amplitude during 30 consecutive sweeps taken 25–30 min after washout of the drug (unless otherwise stated). Spontaneous mEPSCs were collected in the presence of lidocaine (500 μM) at baseline conditions, after 10 min of drug application, and after 20 min of washout of the drug (120 sweeps at each time point, 1 s per sweep) and analyzed using the Mini Analysis program (Synaptosoft, Decatur, GA). The detection limit was set to include only events >10 pA. Data were analyzed statistically with paired Student's *t* test. Analysis of NMDAR-mediated current kinetics was performed comparing averaged EPSCs (15 consecutive sweeps) taken during baseline and 25 min after cocaine washout. Current decay was measured from peak to end and fitted to the following double-exponential equation: $I(t) = I_f \times \exp(-t/\tau_f) + I_s \times \exp(-t/\tau_s)$, where I_f is the amplitude of the fast component, I_s is the amplitude of the slow component, and τ_f and τ_s are their respective decay time constants. To compare decay times between different experimental conditions, we used a weighted mean decay time constant (τ_w), calculated using the following equation: $\tau_w = [I_f/(I_f + I_s)] \times \tau_f + [(I_s/(I_f + I_s)] \times \tau_s$ (Stocca and Vicini, 1998). Data were analyzed statistically with paired Student's *t* test.

Slice homogenates. VTA and hippocampal slices from 21- to 29-d-old Sprague Dawley rats were prepared for electrophysiology as described previously (Ungless et al., 2001). After recovery, slices were transferred to a different chamber with fresh aCSF containing 100 μM picrotoxin and phosphatase inhibitor mixture (to prevent phosphatase activity). Saline or cocaine (1 μM) was added 10 min later for an additional 10 min. After treatments, slices were homogenized in solubilization buffer [25 mM Tris-HCl, pH 7.4, 5 mM EDTA, 5 mM EGTA, 1% deoxycholate, protease inhibitor mixture (Roche Diagnostics, Indianapolis, IN), and phosphatase inhibitor mixture (Sigma)] and analyzed by Western blot. To obtain membranal fractions, samples were centrifuged (5000 rpm for 2 min at 4°C) to remove nuclei and large debris, and total membranes were sedimented (50,000 rpm for 30 min with 70.1 Ti rotors at 4°C), solubilized, and analyzed by Western blot.

Brain homogenates. Sprague Dawley rats (21–29 d of age) were injected intraperitoneally with saline or cocaine (15 mg/kg). The VTA was dissected immediately and frozen in liquid nitrogen. Subcellular fractionation was performed as described previously (Huttner et al., 1983). Briefly, Dounce homogenates of the pellets in ice-cold homogenization buffer (320 mM sucrose, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA, protease inhibitor mixture, and phosphatase inhibitor mixture) were centrifuged at 1000 × *g* to remove nuclei and large debris (P1). The supernatant (S1) was centrifuged at 10,000 × *g* to obtain a crude synaptosomal fraction (P2) and subsequently was lysed hypo-osmotically and centrifuged at 25,000 × *g* to pellet a synaptosomal membrane fraction (LP1). The resulting supernatant (LS1) was centrifuged at 165,000 × *g* to obtain a synaptic vesicle-enriched fraction (LP2). Concurrently, the supernatant (S2) above the crude synaptosomal fraction (P2) was centrifuged at 165,000 × *g* to obtain a cytosolic fraction (S3) and a light membrane fraction (P3). After each centrifugation, the resulting pellet was rinsed briefly with ice-cold homogenization buffer before subsequent fractionations to avoid possible crossover contamination.

Western blot analysis. Protein (25 or 50 μg) from slice or brain homogenates was resolved by 10% SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were probed with the appropriate antibodies, and immunoreactivity was detected with enhanced chemiluminescence (GE Healthcare, Piscataway, NJ) and processed using the Storm system (Molecular Dynamics, Sunnyvale, CA). Digitized images of the bands corresponding to NMDAR subunits or actin were quantitatively analyzed by densitometry with NIH Image 1.61 program providing peak areas. The protein levels or phosphorylation state of the different subunits of the NMDAR were expressed as a percentage of control. Statistical analysis was performed using Student's *t* test (see Fig. 5) and ANOVA (see Fig. 6) for significant differences.

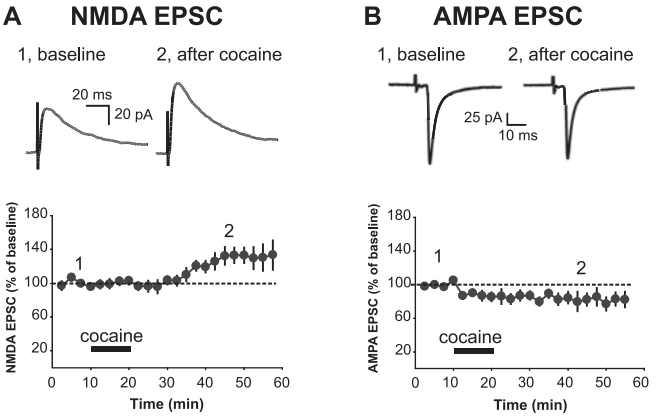

Figure 1. Cocaine causes a delayed enhancement of NMDAR EPSCs. ***A***, Example traces (top) and time-course graph (bottom; $n = 6$) showing the effects of cocaine superfusion (1–3 μM; 10 min) on mean NMDAR EPSCs in cells voltage clamped at +40 mV. ***B***, Example traces (top) and time-course graph (bottom; $n = 6$) showing the effects of cocaine on AMPAR-mediated EPSCs (AMPAR EPSCs) recorded in cells voltage clamped at −70 mV. Averaged sample EPSCs were taken 5 min before (1) and 25 min after (2) cocaine application. Horizontal bars represent the time that cocaine was included in the superfusion medium. Error bars represent SEM.

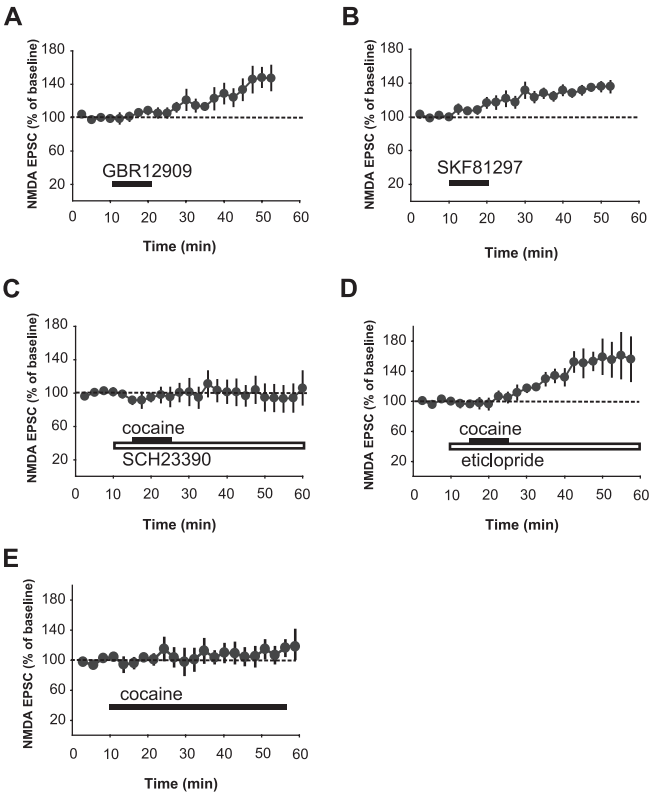

Figure 2. Cocaine enhances NMDAR EPSCs via activation of DA D_1/D_5 receptors. ***A***, Time course of mean NMDAR EPSCs shows that application of the specific DA reuptake inhibitor GBR12909 (10 nM; 10 min; $n = 6$) mimics the effect of cocaine on NMDAR EPSCs. ***B***, The D_1/D_5 agonist SKF81297 (10 μM; 10 min; $n = 8$) increases NMDAR EPSCs without the delay seen with cocaine and GBR12909. ***C***, The DA D_1/D_5 receptor antagonist SCH23390 (10 μM; $n = 4$) inhibited cocaine enhancement of NMDAR EPSCs. ***D***, The DA D_2-like receptor antagonist eticlopride (3 μM; $n = 6$) accelerates the effect of cocaine on NMDAR EPSCs but does not affect the magnitude. ***E***, No enhancement of NMDAR EPSCs develops during continuous application of cocaine (1–3 μM; 50 min; $n = 4$). Horizontal bars represent the time that drugs were included in the superfusion medium. Error bars represent SEM.

Results

Effects of cocaine on EPSCs

EPSCs were evoked by electrically stimulating afferents while DA cells were voltage clamped at +40 mV. At this membrane potential, EPSCs are mediated by both AMPARs and NMDARs (Ungless et al., 2001). Cocaine did not affect the EPSC amplitude during its application (1–3 μM; 10 min) but produced a delayed increase starting 15–20 min after termination of cocaine superfusion (132 ± 9% of baseline; 45–50 min; $n = 6$; $p < 0.01$) (Fig. 1*A*). This increase was attributable to an effect on the NMDAR EPSCs, because the EPSC amplitude was increased when measured 20 ms after the stimulus artifact, when the AMPAR-mediated component of the EPSCs (AMPAR EPSCs) is negligible (Ungless et al., 2001). Indeed, cocaine caused no change when a measurement was made on the rising phase of the EPSC, a time point at which AMPAR EPSCs predominate (data not shown). Moreover, to test specifically for possible effects of cocaine on AMPAR EPSCs, in a separate set of experiments, cells were voltage clamped at −70 mV, a membrane potential at which NMDARs do not contribute to EPSCs. Cocaine caused a small decrease of AMPAR EPSCs under these conditions (82 ± 7%; $n = 6$; $p < 0.05$) (Fig. 1*B*). Because a presynaptic enhancement of glutamate release should equally affect both AMPAR EPSCs and NMDAR EPSCs, these initial observations indicate that the effect of cocaine on NMDAR EPSCs is postsynaptic.

Effects of dopamine reuptake inhibitors, agonists, and antagonists

Cocaine exerts a wide variety of effects in the CNS, because it inhibits the reuptake of DA, serotonin, and noradrenaline (Kuhar et al., 1991). In fact, microdialysis studies have shown that cocaine elevates the extracellular levels of all three transmitters in the VTA (Reith et al., 1997). Thus, the cocaine-induced enhancement of NMDAR EPSCs could be attributable to an increased level of any of these transmitters, alone or in combination. We first examined the potential role of DA in mediating the effects of cocaine by applying the specific DA reuptake inhibitor GBR12909 (10 nM for 10 min). This manipulation mimicked the effect of cocaine on NMDAR EPSCs (133 ± 12%; $n = 6$; $p < 0.01$) (Fig. 2*A*), suggesting that the effect of cocaine is caused by inhibition of reuptake of somatodendritically released DA and, indirectly, by stimulation of DA receptors.

DA neurons in the midbrain have been shown to express DA D_5 receptors (Ciliax et al., 2000; Khan et al., 2000), suggesting that cocaine-induced increase in extracellular DA results in stimulation of postsynaptic DA D_5 receptors. Presently, there are no pharmacological agents available to distinguish D_1 receptors from D_5 receptors. However, neither D_1 receptor mRNA nor protein has been found in DA cell bodies (Weiner et al., 1991; Yung et al., 1995); therefore, any action of mixed D_1/D_5 agents in midbrain DA cells is likely mediated via DA D_5 receptors. Figure 2*B* shows that bath application of the DA D_1/D_5 receptor agonist SKF81297 (10 μM; 10 min) enhanced NMDAR EPSCs (132 ± 5%; $n = 8$; $p < 0.01$). Moreover, pretreatment of slices with the DA D_1/D_5 receptor antagonist SCH23390 (10 μM) prevented the potentiation of NMDAR EPSCs by cocaine (97 ± 12%; $n = 4$; $p > 0.05$) (Fig. 2*C*), suggesting that stimulation of D_5 receptors is both necessary and sufficient for cocaine to induce an increase of NMDAR EPSCs.

Interestingly, the effect on NMDAR EPSCs induced by SKF81297 developed during the drug application and did not require a period of washout (Fig. 2*B*). Combined with the fact that cocaine slightly inhibited AMPAR EPSCs (Fig. 1*B*), it seems that the effect of cocaine on NMDAR EPSCs is the sum of differ-

ent effects on glutamate neurotransmission elicited by cocaine. Thus, the delay in cocaine-induced potentiation of NMDAR EPSCs could be a result of the previously reported presynaptic inhibition of glutamate release mediated either by DA D_2 receptors (Koga and Momiyama, 2000) or 5-hydroxytryptamine (5-HT)$_2$ receptors (Jones and Kauer, 1999). This raised the possibility that, initially, the effect of cocaine on NMDAR EPSCs is masked by inhibition of glutamate release, an effect that wanes as NMDAR currents grow stronger. If this idea is correct, the enhancement of NMDAR EPSCs elicited by cocaine should not be detected during continuous cocaine application. As predicted, when cocaine was applied continuously (50 min) and no washout was applied, there was no effect on NMDAR EPSCs (104 ± 10%; $n = 4$; $p > 0.05$) (Fig. 2*E*). Because cocaine and GBR12909 produced similar effects, we reasoned that inhibition of glutamate release could, at least partially, be mediated via dopamine. Therefore, we pretreated slices with the DA D_2 receptor antagonist eticlopride (3 μM; 5 min) followed by the application of cocaine in continued presence of eticlopride. Under these conditions, the cocaine-induced enhancement of NMDAR EPSCs developed earlier with a significant enhancement of NMDAR EPSCs within 5 min after cocaine superfusion (111 ± 5% at 25–30 min, 5 min after cocaine superfusion; 153 ± 11% at 45–50 min; $n = 6$; $p < 0.01$) (Fig. 2*D*). Together, these results suggest that DA D_5 receptor activation during exposure to cocaine leads to a slowly developing enhancement of NMDAR EPSCs and that simultaneous activation of presynaptic DA D_2 receptors can delay this effect, likely by reducing glutamate release.

Effects of D_1-like agonists on AMPAR EPSCs and mEPSCs

Microdialysis data have shown that D_1-like agonists increase the extracellular level of glutamate in the VTA (Kalivas and Duffy, 1995). However, as mentioned above, more recent studies have reported that both presynaptic serotonin and dopamine receptors can reduce glutamate synaptic transmission in the VTA (Jones and Kauer, 1999; Koga and Momiyama, 2000). Other studies suggest that D_1-like receptor stimulation in the VTA leads to a delayed increase of extracellular glutamate, although the increase in glutamate was not attributable to synaptic spillover (Wolf and Xue, 1998, Wolf et al., 2000). Therefore, to further ensure that the effect of cocaine and the D_5-like agonist on NMDAR EPSCs was truly postsynaptic, we both tested the effect of the D_5-like agonist on AMPAR EPSCs evoked by paired-pulse stimulation and measured the frequency and amplitude of miniature EPSCs (mEPSCs). These are standard methods to determine the locus of a change in synaptic efficacy (Dobrunz and Stevens, 1997; Malenka and Nicoll, 1999). During application, the D_1/D_5 agonist SKF81297 (10 μM) slightly depressed the amplitude of the first EPSC in the paired-pulse protocol (94 ± 3%; $n = 6$; $p < 0.05$), and although the second EPSC was not significantly affected (96 ± 2%; $n = 6$; $p > 0.05$), the depression of the first response was not large enough to cause a significant change in the ratio during and after drug superfusion (basal, 0.76 ± 0.08; SKF81297, 0.79 ± 0.08; 25 min after SKF81297 washout, 0.80 ± 0.06; $n = 5$; $p > 0.05$) (Fig. 3*A*).

Furthermore, SKF81297 (10 μM) caused a slight but significant reduction of mEPSC frequency (basal, 0.51.± 0.07 Hz; SKF81297, 0.44 ± 0.08 Hz; $p < 0.01$; $n = 5$), although this effect was not significant after 20 min (0.50 ± 0.06 Hz; $p > 0.05$; $n = 5$) (Fig. 3*B*). Together with the fact that SKF81297 had no effect on the amplitude of mEPSCs (Fig. 3*B*), these results suggest that presynaptic dopamine D_1-like receptors may exert a slight inhibitory effect on glutamate release. Our results might appear in conflict with the microdialysis study by Kalivas and Duffy (1995) reporting a D_1/D_5-dependent increase of glutamate release in the VTA. However, our hypothesis is that the increase in glutamate release observed by Kalivas and Duffy (1995) might be a consequence of the D_5-dependent increase of VTA NMDAR function, which in turn could increase DA release in brain regions such as the prefrontal cortex (PFC), increase firing of PFC neurons via DA receptor activation (Tseng and O'Donnell, 2004) and thus enhance glutamate release in the VTA from PFC afferents.

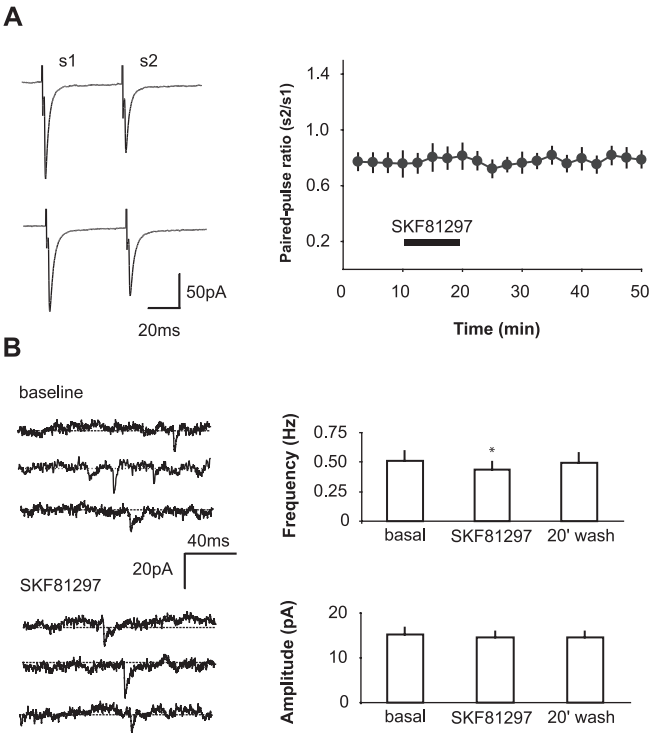

Figure 3. DA D_1/D_5 receptor activation transiently decreases mEPSC frequency. ***A***, SKF81297 superfusion (10 μM; 10 min) had no significant effect on paired-pulse ratio (right; $n = 6$). Sample traces show EPSCs evoked by paired pulses delivered 50 ms apart in cells voltage clamped at −70 mV, before (top left) and during (bottom left) drug superfusion. ***B***, SKF81297 superfusion (10 μM; 10 min) had no effect on mEPSC amplitude but transiently decreased mEPSC frequency. Example mEPSC traces recorded in lidocaine before (top left) and during (bottom left) drug superfusion. The bar histograms represent mean mEPSC frequency (top right) and amplitude (bottom right) ± SEM. The asterisk indicates significant differences between treatment and control samples (*$p < 0.05$; Student's *t* test; $n = 5$).

DA D_5 receptors are Gαs-protein-coupled receptors that signal via activation of adenylate cyclase to increase the formation of cAMP, which in turn activates PKA (Sunahara et al., 1991; Tiberi et al., 1991). Thus, it was expected that interference with the cAMP/PKA pathway would inhibit the effect of cocaine on NMDAR EPSCs. Accordingly, when the inhibitory cAMP analog, Rp-cAMPs (100 μM), was included in the pipette, the effect of cocaine on NMDAR EPSCs was blocked (95 ± 10%; $n = 4$; $p > 0.05$) (Fig. 4*A*). To specifically test the role of PKA, the ability of cocaine to increase NMDAR-mediated EPSCs was determined by adding the PKA inhibitor PKI(6-22)-amide (20 μM) to the recording pipette. Under these recording conditions, there was again no effect of cocaine on the NMDAR EPSCs (89 ± 17%; $n = 4$; $p > 0.05$) (Fig. 4*B*). These results suggest that the effect of cocaine on NMDAR EPSCs is mediated via activation of the cAMP/PKA signaling pathway and also provide even stronger evidence that the effect of cocaine on NMDAR is postsynaptic, because the PKA inhibitor was administered into the recorded

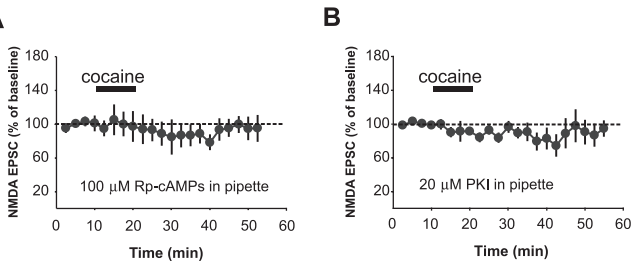

Figure 4. The enhancement of NMDAR EPSCs by cocaine involves the cAMP/PKA signaling pathway. ***A***, The effect of cocaine on NMDAR EPSCs was inhibited when the PKA inhibitor Rp-cAMPs (100 μM; $n = 4$) was included in the recording pipette. ***B***, The effect of cocaine was also inhibited when the membrane-impermeable PKA inhibitor PKI(6-22)-amide (20 μM; $n = 4$) was included in the recording pipette. Horizontal bars represent the time that cocaine was included in the superfusion medium. Error bars represent SEM.

cell via the recording pipette, and the compound is membrane impermeable.

Acute cocaine exposure induces changes in protein level and distribution of NR1 and NR2B subunits in the VTA

Next, we conducted a set of biochemical experiments to identify a possible mechanism for the enhancement of NMDAR EPSCs after acute cocaine exposure. The NMDARs are heteromers composed of an obligatory NR1 subunit together with modulatory NR2A–NR2D subunits (Sucher et al., 1996; Cull-Candy and Leszkiewicz, 2004). Therefore, the observed postsynaptic increase in NMDAR activity after cocaine could be the result of a subunit-specific increase in the expression, synaptic localization, and/or function of NMDARs. We started addressing these possibilities by examining changes in protein levels of NMDAR subunits in VTA slices, which were treated with cocaine (1 μM) for 10 min, using Western blot analysis. As shown in Figure 5*A*, we observed a significant increase in the immunoreactivity of both NR1 and NR2B subunits in VTA total homogenates in cocaine-treated (NR2B, 187 ± 63%; NR1, 134 ± 5%; $n = 6$; $p < 0.05$; all values are presented as percentage of control) but not saline-treated slices. Furthermore, the observed increase in the immunoreactivity of NR1 and NR2B was specific, because no change in the protein levels of NR2A and NR2C was detected after acute cocaine exposure (Fig. 5*A*). Next, we determined whether the increase in NR1 and NR2B was specific to the VTA by measuring the immunoreactivity of the NMDAR subunits in hippocampal slices. Our results show that there was no change in protein levels of NR1 or NR2B in hippocampal slices treated with cocaine (Fig. 5*A*). Next, we determined whether the cocaine-induced increase in the amount of NR2B in the VTA is attributable to an increase in new protein synthesis. To do so, we pretreated VTA slices with the protein synthesis inhibitor anisomycin (20 μM) 60 min before 10 min incubation with cocaine (1 μM). As shown in Figure 5*B*, anisomycin pretreatment blocked the cocaine-induced increase of NR2B (NR2B cocaine, 189 ± 49%; cocaine plus anisomycin, 123 ± 16%; anisomycin, 88 ± 16%) and NR1 without affecting the basal levels of these proteins, indicating that acute cocaine exposure results in an increase in newly synthesized receptor subunits in a region- and subunit-specific manner. Activation of the cAMP/PKA pathway has been implicated as playing a role in the targeting of NMDAR subunits to the synapse in cultured hippocampal neurons (Crump et al., 2001). Based on these findings, we hypothesized that the cocaine-induced increase in NMDAR EPSCs after DA D_5 receptor stimulation and activation of PKA was a result of an increased number of NR2B-containing NMDARs in the synaptic membrane. To test this hypothesis, VTA slices were incubated with 1 μM cocaine for 10 min and homogenized either immediately or 30 min after washout of cocaine (when maximal increase in NMDAR EPSCs was observed), and the levels of NR1, NR2A, and NR2B in the membrane fraction were determined. As shown in Figure 5*C*, 10 min of exposure to cocaine induced significant increase in the level of NR2B (152 ± 7%; $n = 3$; $p < 0.05$), which remained elevated after washout of cocaine (148 ± 7%; $n = 3$; $p < 0.05$), suggesting that the NR2B-containing NMDARs are indeed distributed to the membrane. Previous studies suggested that in striatal neurons, DA D_1-like receptor activation resulted in redistribution of NMDAR subunits to the synaptic membrane by a mechanism that requires Fyn tyrosine kinase (Dunah and Standaert, 2001; Dunah et al., 2004). We previously showed that activation of the cAMP/PKA pathway leads to the phosphorylation of the NR2B subunit by Fyn (Yaka et al., 2003). Based on these results, we hypothesized that the observed increase in membranal NR2B is mediated by Fyn phosphorylation of NR2B. Therefore, we tested whether cocaine incubation of VTA slices alters the phosphorylation state of the subunit. However, acute exposure of VTA slices to cocaine did not result in a change in the phosphorylation state of the NR2B subunit (Fig. 5*D*). Next, we set out to determine whether the observed increase in the membranal expression of NMDAR in the VTA slice preparation also occurs *in vivo*. Rats were injected intraperitoneally with 15 mg/kg cocaine or saline; 30 min after injection, the VTA was dissected, and subcellular fractionation was performed to isolate the different membrane compartments, which were verified by using two synaptic markers, synaptophysin and PSD-95 (presynaptic and postsynaptic markers, respectively) (Fig. 6*B*), as described previously (Huttner et al., 1983). Indeed, acute injection of cocaine resulted in an increase in the levels of both NR1 and NR2B subunits in the synaptosomal (LP1) and vesicle-enriched (LP2) fractions, which contain the postsynaptic density and synaptic vesicles, respectively (LP1 NR1, 130 ± 3%; NR2B, 127 ± 3%; LP2 NR1, 147 ± 11%; NR2B, 160 ± 4%; $n = 3$; $p < 0.05$ relative to saline) (Fig. 6*A*) [Dunah and Standaert (2001) and references therein]. A decrease of NR1 and NR2B was found in the light membrane fraction (P3) (NR1, 41 ± 3%; NR2B, 39% ± 11; $n = 3$; $p < 0.05$ relative to saline), containing cellular organelles such as endoplasmic reticulum and Golgi, and no changes in the levels of NR2A or NR2C were observed (Fig. 6*A*). These data suggest that there is a specific increase (or movement) of the NR1 and NR2B subunits of the NMDAR to the synaptic membranes 30 min after an *in vivo* cocaine injection. However, no change in the cellular distribution of AMPAR subunit GluR1 was detected under the same experimental conditions (Fig. 6*B*).

Pharmacological and biophysical properties of cocaine-potentiated NMDAR EPSCs

In a first series of experiments, we wanted to test whether NR2B-containing NMDARs were necessary for cocaine-induced potentiation. Figure 7*A* shows that the presence of the selective NR2B antagonist ifenprodil (3 μM) (Williams, 1993) in the perfusion medium completely prevented cocaine-induced enhancement of NMDAR EPSCs (87 ± 23%; $n = 4$; $p > 0.05$). These data suggest that NR2B-containing NMDARs are necessary for the induction of cocaine-induced potentiation of NMDAR-mediated currents.

Early in development, NR2B subunits predominate, and progressively, NR2A subunits are incorporated in functional NMDARs as rodents become older (Watanabe et al., 1992; Monyer et al., 1994). NMDARs can contain diheteromeric NR1/

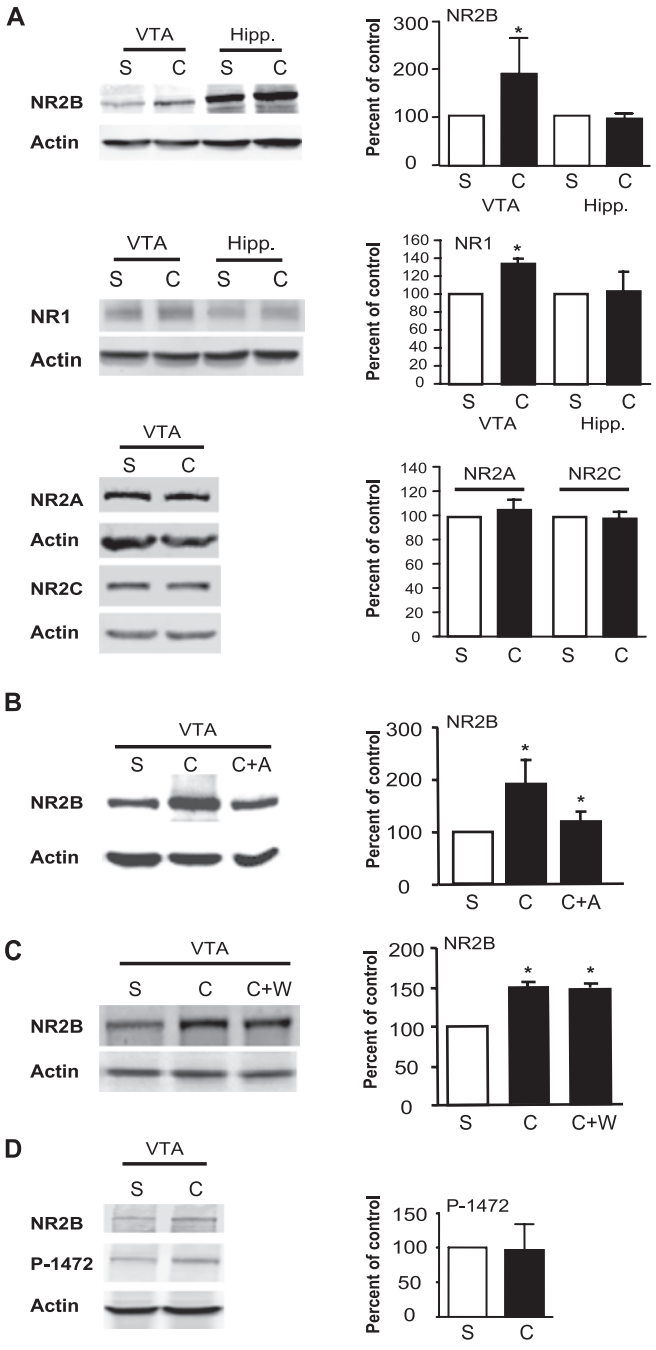

Figure 5. Acute exposure to cocaine induces an increase in NR1 and NR2B subunit immunoreactivity in the VTA. ***A***, VTA and hippocampal slices (Hipp.) were incubated without [saline (S)] or with 1 μM cocaine (C) for 10 min. After treatment, slices were homogenized, and samples (25 μg) were resolved by SDS-PAGE. The levels of NR2B (top), NR1 (middle), and NR2A and NR2C (bottom) were detected using specific antibodies and normalized to actin. The bar histograms represent normalized levels of NMDAR subunits plotted as percentage of control ± SD of six (NR2B), four (NR1), and three (NR2A and NR2C) independent experiments. Asterisks indicate significant differences between treatment and control samples (*$p < 0.05$; Student's *t* test). ***B***, VTA slices were preincubated for 60 min with the protein synthesis inhibitor anisomycin (20 μM) followed by 10 min of 1 μM cocaine (C+A) or with anisomycin alone (A). Slices were also incubated without or with 1 μM cocaine for 10 min. After treatment, slices were homogenized, and samples (25 μg) were resolved by SDS-PAGE. The levels of NR1 and NR2B were detected using specific antibodies and normalized to actin. The bar histograms represent normalized levels of NR2B plotted as percentage of control ± SD of three independent experiments. Asterisks indicate significant differences between cocaine-treated slices and control slices or cocaine and anisomycin plus cocaine slices (*$p < 0.05$; Student's *t* test). ***C***, VTA slices were treated without or with 1 μM cocaine for 10 min or with 1 μM cocaine for 10 min followed by 30 min washout of cocaine (C+W). After treatments, the level of membranal NR2B in the samples (50 μg) was determined as in ***A***. The bar histogram of normalized NR2B levels is plotted as percentage of control ± SD of three independent experiments (*$p < 0.05$; Student's *t* test). ***D***, VTA slices were treated as in ***A***. Samples (25 μg) were resolved by SDS-PAGE, and the level of NR2B phosphorylation was detected using site-specific phospho-NR2B antibodies (P-1472) normalized to total NR2B. The bar histogram of normalized P-1472 NR2B levels is plotted as percentage of control ± SD of six independent experiments.

NR2 pairs or can be expressed as triheteromeric complexes such as NR1/NR2A/NR2B (Sheng et al., 1994; Chazot and Stephenson, 1997; Luo et al., 1997; Stocca and Vicini, 1998; Tovar and Westbrook, 1999; Prybylowski et al., 2002; Dunah and Standaert, 2003). These triheteromeric NMDARs appear to display different pharmacological properties when compared with diheteromeric NR1/NR2B-containing NMDARs, such as decreased sensitivity to NR2B antagonists (Vicini et al., 1998; Tovar and Westbrook, 1999). Our biochemical results show that acute cocaine results in an increased expression of NR2B subunits in the VTA, which could be present in the membrane in the form of either "pure" NR1/NR2B-containing NMDARs or triheteromers formed by NR1, NR2A, and NR2B subunits.

Thus, in our next series of experiments, we assessed the relative contribution of NR2B subunits in mediating NMDAR responses in the VTA. Three different concentrations of ifenprodil (0.3–10 μM) (Williams, 1993) were tested in control slices and in slices pretreated with cocaine, 30 min after washout of the drug, once cocaine induced a steady potentiation. If cocaine causes an increase of dimeric NR1/NR2B NMDARs, it is expected that ifenprodil would produce a larger inhibition of NMDAR-mediated responses in cocaine-treated slices than in control slices. However, we were not able to detect any significant difference between the sensitivity to ifenprodil under control conditions (87 ± 6, 64 ± 5, and 50 ± 5% at 0.03, 0.3, and 3 μM, respectively; $n = 4$) versus after acute cocaine application (93 ± 2, 65 ± 2, and 51 ± 3% at 0.3, 3, and 10 μM, respectively; $n = 11$; $p > 0.05$; two-way ANOVA) (Fig. 7*B*). To further confirm this finding, we tested the effect of Ro25-6981, another NR2B antagonist that is more selective and potent than ifenprodil (Fischer et al., 1997). In agreement with our previous results, we were not able to detect any significant increased efficacy of the NR2B antagonist in inhibiting NMDAR currents after cocaine (88 ± 2, 61 ± 5, and 34 ± 5% at 0.005, 0.5, and 5 μM, respectively; $n = 7$) when compared with control slices (80 ± 4, 58 ± 5, and 21 ± 4% at 0.005, 0.5, and 5 μM, respectively; $n = 5$; $p > 0.05$; two-way ANOVA) (Fig. 7*B*). Finally, consistently with our Western blot analysis, when we tested the putative NR2A antagonist NVP-AAM077 (0.004–4 μM) (Auberson et al., 2002), we observed a similar dose-dependent inhibition of NMDAR-mediated responses both in control slices and in slices pretreated with cocaine (data not shown), although we need to point out that NVP-AAM077 might also bind NR2C-containing receptors (Feng et al., 2004). Together, our biochemical results suggest that acute cocaine causes increased expression of NR2B-containing NMDARs, specifically at the synaptic membrane, whereas our pharmacological results seem to suggest increased presence of functional NR1/NR2A/NR2B complexes, which are less sensitive to ifenprodil and Ro25-6981 antagonism, rather than dimeric NR1/NR2B NMDARs.

To provide additional functional evidence for our findings, we analyzed the kinetics of NMDAR EPSCs before exposing slices to cocaine (1–3 μM) and 25 min after cocaine exposure. NR2 subunits confer the distinct pharmacologic and kinetic properties of NMDARs (Yamakura and Shimoji, 1999; Cull-Candy and Lesz-

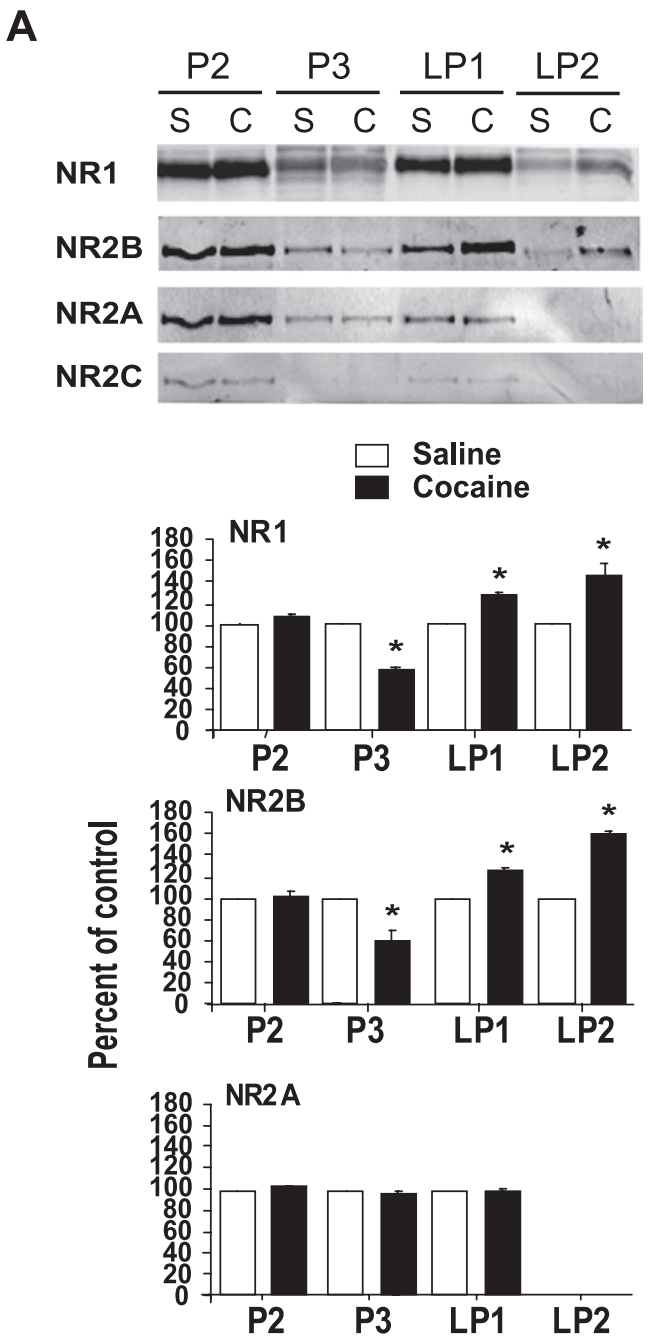

Figure 6. Systemic administration of cocaine induces a redistribution of NR1 and NR2B subunits in the VTA *in vivo*. ***A***, Levels of NMDAR subunits NR1, NR2B, NR2A, and NR2C in the VTA in the crude synaptosomal (P2), light membrane (P3), synaptosomal membrane (LP1), and synaptic vesicle-enriched (LP2) fractions from saline (S) or 30 min after intraperitoneal injection of cocaine (C; 15 mg/kg), resolved by SDS-PAGE (50 μg per lane). The histograms depict the relative levels of NR1, NR2B, and NR2A proteins, given as percentage of the control samples. Data are means ± SEM of three independent experiments. Asterisks indicate significant differences between treatment and control samples (*$p < 0.05$; ANOVA). ***B***, Animals were treated as in ***A***, and levels of GluR1, PSD-95, and synaptophysin (Syn.) in the different subcellular membranal fractions (50 μg per lane) were detected using specific antibodies as indicated. No change in protein levels was found after normalization to anti-actin antibodies (data not shown).

kiewicz, 2004). Dimeric receptors containing NR1/NR2B have slower offset kinetics than receptors built of NR1/NR2A, whereas triheteromeric receptors containing both NR2A and NR2B subunits are characterized by intermediate properties, such as reduced sensitivity to selective NR2B antagonists but slow offset kinetics dominated by the presence of NR2B subunit (Plant et al., 1997; Vicini et al., 1998; Tovar and Westbrook, 1999). A previous study identified functionally different NMDARs in the ventromedial and the dorsolateral striatum on the basis of their different decay time kinetics (Chapman et al., 2003), related to previously observed regional difference in the expression of NR2A and NR2B subunits (Standaert et al., 1994).

Figure 7*C* shows that decay time kinetics of NMDAR EPSCs recorded at +40 mV in VTA neurons from young rats [postnatal day 21 (P21) to P29] were fitted with the double-exponential equation; to compare decay times between different experimental conditions, we calculated a weighted decay time constant (τ_w) derived from the weighted mean of the fast and the slow time constants. Bath application of cocaine (10 min; 1–3 μM) significantly slowed the time course of NMDAR current decay (τ_w baseline, 0.043 ± 0.003 s; τ_w 25 min after cocaine washout, 0.067 ± 0.004 s; $n = 22$; $p < 0.01$) (Fig. 7*C*), consistent with an increased presence of NR2B-containing receptors. A previous report (Plant et al., 1997) demonstrated that septal neurons in rat forebrain slices contained heteromers of NR2A and NR2B subunits, based on sensitivity to ifenprodil and on NMDAR EPSC decay time kinetics dominated by the NR2B subunit. Therefore, our data suggest that the cocaine-induced increased levels of NR1 and NR2B subunits in the membrane are likely related to increased insertion of triheteromeric NMDARs in VTA DA neurons.

Discussion

A number of studies have shown that development of behavioral sensitization to cocaine involves effects on glutamatergic transmission in the VTA [Wolf (1998) and references therein]. Here, cocaine was primarily found to cause a delayed increase in NMDAR EPSCs. The DA reuptake inhibitor GBR12909 mimicked the effect of cocaine, suggesting that the effect is attributable to an increased extracellular level of DA and indirectly to stimulation of DA receptors. Accordingly, cocaine-induced potentiation of NMDAR EPSCs was prevented by the DA D_1/D_5 receptor antagonist SCH23390 and was mimicked by DA D_1/D_5 agonist SKF81297. Thus, stimulation of a D_1/D_5 receptor is both necessary and sufficient for the enhancement of NMDAR EPSCs by cocaine. The fact that NMDAR EPSCs, but not AMPAR EPSCs, were increased suggests that the effect is caused by a postsynaptic mechanism. Anatomical evidence suggests that any postsynaptic effect of mixed D_1/D_5 agents in midbrain DA cells is likely mediated via DA D_5 receptors. D_5 receptors are present on cell bodies in the VTA and substantia nigra (Ciliax et al., 2000; Khan et al., 2000), and they localize postsynaptically on tyrosine hydroxylase-positive neurons (Kahn et al., 2000). In contrast, no

cell body staining was reported with D_1 receptor antibodies, although other neuronal structures were stained (Yung et al., 1995). Hence, the D_1 receptor protein found in the VTA is very unlikely to reside on DA cells, suggesting that the postsynaptic DA D_1/D_5 receptor-mediated enhancement of NMDAR EPSCs described in the present study is mediated via the D_5 receptors. Nevertheless, given the lack of commercially available D_5 selective ligands, we needed to provide additional indirect evidence to substantiate the postsynaptic nature of the effect of cocaine. First, we excluded any involvement of presynaptic DA D_1-like receptors by showing no evidence for a D_1-like mediated facilitation of glutamate release. In fact, the D_1/D_5 agonist SKF81297 increased NMDAR EPSCs but not AMPAR EPSCs. Moreover, the D_1/D_5 agonist caused a small and transient decrease of mEPSC frequency. Second, when the recorded cell was loaded with the cAMP/PKA inhibitors Rp-cAMPs and PKI, the effect of cocaine was prevented. These results, albeit indirectly, strongly suggest that the cocaine-induced increase in NMDAR EPSCs is a result of stimulation of postsynaptic D_5 receptors.

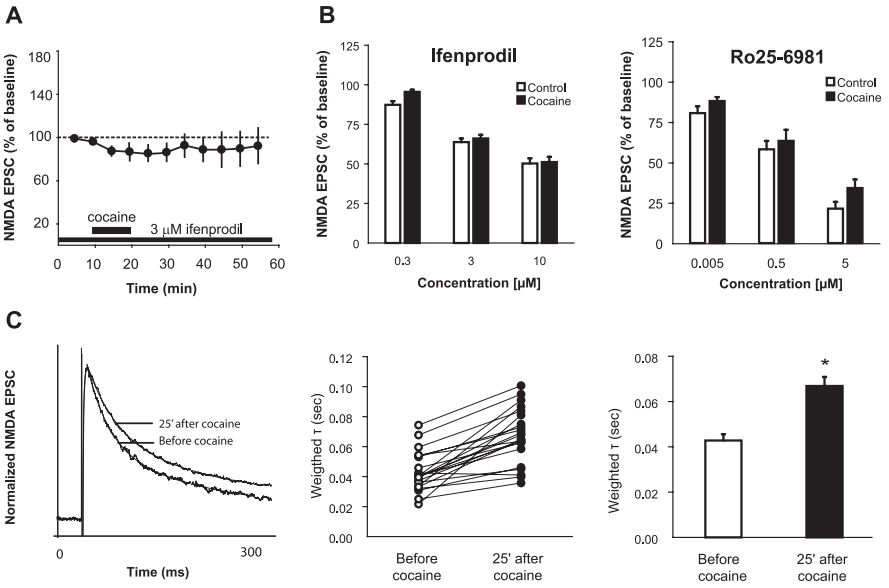

Figure 7. Acute cocaine increases expression of NR2B subunits likely in triheteromeric NMDAR complexes. ***A***, NMDAR EPSC enhancement by cocaine requires activation of NR2B receptors. Time course of mean NMDAR EPSCs shows that in the continuous presence of 3 μM ifenprodil, cocaine did not cause an enhancement of NMDAR EPSCs ($n = 4$). The horizontal bar represents the time that cocaine was included in the superfusion medium. ***B***, NR2B-specific antagonists ifenprodil (0.003–10 μM; left) and Ro25-6981 (0.005–5 μM; right) inhibit NMDAR EPSCs to the same extent in control slices (white bars; ifenprodil, $n = 4$; Ro25-6981, $n = 5$) and in slices pretreated with cocaine (black bars; ifenprodil, $n = 11$; Ro25-6981, $n = 7$) ($p > 0.05$; two-way ANOVA). ***C***, Analysis of NMDAR EPSC decay time suggests a cocaine-induced switch toward a higher synaptic NR2B/NR2A ratio. Left, Sample traces of NMDAR EPSCs before and after cocaine recorded at +40 mV and normalized to 100 pA for comparison. Each trace is the average of 15 consecutive sweeps. Middle, Summary scatter plots of τ_w (weighted decay time constant) of NMDAR EPSCs from individual VTA cells before (open circle) and 25 min after (filled circle) cocaine superfusion. Right, Mean decay time constant value of NMDAR EPSCs increases significantly after cocaine application (*$p < 0.005$; Student's *t* test; $n = 22$). Error bars represent SEM.

The cocaine-induced enhancement of NMDAR EPSCs was delayed and required a period of washout to develop. Because the effect of cocaine developed faster in the presence of the DA D_2 receptor antagonist eticlopride, we hypothesized a D_2 receptor-mediated inhibition of glutamate release that would account also for the small but significant decrease in AMPAR EPSCs by cocaine (Koga and Momiyama, 2000). However, the lack of an inhibitory effect of cocaine on NMDAR EPSCs in the presence of SCH23390 indicates that other mechanisms may be involved. Indeed, the transient reduction by DA D_1/D_5 agonist SKF81297 of mEPSC frequency is indicative of presynaptic inhibition of glutamate release. Thus, we propose that in the *in vitro* slice preparation, cocaine produces two acute DA-mediated effects on glutamatergic transmission in the VTA: potentiation of NMDAR-mediated responses via postsynaptic DA D_5 receptors and, at the same time, inhibition of glutamate release via presynaptic DA D_1/D_5 and D_2 receptors. It should be noted that presynaptic inhibition of glutamate release via 5-HT receptors (Jones and Kauer, 1999) could also partially contribute to the effects observed here. An interesting possibility is that, *in vivo*, the enhanced effect of cocaine on NMDAR function would not be unmasked until the withdrawal phase. It is known that NMDARs in the VTA regulate burst firing of dopamine neurons (Chergui et al., 1993). Dopamine neurons fire bursts of action potentials in response to stimuli of motivational value (see Schultz, 1998). Our data indicate that during withdrawal from cocaine, when NMDAR function is increased, dopamine neurons would be more likely to fire in bursts and falsely give salience to otherwise neutral stimuli.

We observed that cocaine exposure resulted in increased levels of the NMDAR subunits NR1 and NR2B in the total homogenates from VTA slices that appear to be a result of newly synthesized subunits. Because the observed increase in NMDAR subunits is very rapid, it might be attributable to activation of the translation machinery locally at the dendritic domain, in a transcription-independent manner, as was shown previously for PSD-95 in hippocampal slices after short stimulation with insulin (Lee et al., 2005). However, this hypothesis requires additional investigation. Our data show that stimulation of dopamine receptors as a result of acute cocaine exposure and PKA pathway activation triggered the processes that lead to increased levels of synaptic NR2B-containing NMDARs. Accordingly, a recent study shows that in striatal slices, D_1 receptor activation leads to rapid enhancement of dendritic localization, clustering, and surface expression of NR2B and NR1 NMDA subunits, regulated by tyrosine phosphatases and kinases (Hallett et al., 2006). Furthermore, in striatal slices, a 10 min application of the D_1/D_5 receptor agonist SKF82958 caused a redistribution of NMDAR subunits, including NR1 and NR2B, to the synaptic membrane (Dunah and Standaert, 2001), by a mechanism that requires Fyn kinase (Dunah et al., 2004). We previously showed that activation of the cAMP/PKA pathway leads to the phosphorylation of the NR2B subunit by Fyn, resulting in enhanced NMDAR EPSCs (Yaka et al., 2003). However, we did not detect changes in NR2B phosphorylation after 10 min of cocaine treatment, possibly because NR2B phosphorylation is a rapid and transient process, which, under the current conditions, we failed to detect. Furthermore, we studied the distribution of NMDAR subunits in the VTA after a single cocaine injection. Similarly to our *in vitro* findings, cocaine increased the amount of NR1 and NR2B in the fractions containing synaptic membranes and synaptic vesicles, whereas a significant decrease was observed in the fraction containing intracellular organelles, suggesting a movement of these subunits toward the synapse.

Analysis of NMDAR decay time kinetics shows that they were significantly slower after cocaine exposure, further suggesting an increased membrane expression of NR2B-containing NMDARs in the VTA. In addiction, our pharmacological experiments showed that there is no increased sensitivity of NMDARs to selective NR2B subunit antagonists after acute cocaine compared with controls. These results might be interpreted as an indication of an increased number of NMDARs containing both NR2A and NR2B subunits at the synaptic level induced by cocaine. Indeed, triheteromeric NMDARs composed of NR1/NR2A/NR2B are characterized by specific properties that distinguish them from pure NR1/NR2A- or NR1/NR2B-containing NMDARs, such as decreased sensitivity to NR2B antagonist and slow decay time kinetics (Plant et al., 1997; Vicini et al., 1998; Tovar and Westbrook, 1999; Hatton and Paoletti, 2005). Thus, we suggest that potentiation of NMDAR EPSCs after cocaine is attributable to incorporation of more NR2B-containing synaptic NMDARs, although other possible mechanisms and NMDA subunits may contribute. Although there is evidence that NR2D-subunit mRNA (Monyer et al., 1994) and protein (Dunah et al., 1996) might be expressed at low levels in the midbrain, we did not detect NR2D immunoreactivity in the VTA. The lack of NR2D signal could be attributable to poor selectivity of the antibodies used or to a very low level of NR2D that we failed to detect. Therefore, we cannot completely rule out the possibility that triheteromeric receptors containing NR2D subunits might be formed as well (Dunah et al., 1998; Jones and Gibb, 2005). Furthermore, although our biochemical results speak in favor of activation of NMDAR trafficking as the primary mechanism responsible for the cocaine-dependent potentiation of NMDAR currents, lateral movement of extrasynaptic receptors may also be a contributing factor (Tovar and Westbrook, 2002).

Our data provide a plausible explanation as to how cocaine causes NMDAR-dependent LTP in midbrain DA neurons (Ungless et al., 2001). Recent evidence has shown that blockade of D_1/D_5 receptor inhibits the AMPAR/NMDAR ratio increase on VTA neurons produced by *in vivo* cocaine exposure (Dong et al., 2004), supporting our hypothesis for a role of a D_5-dependent potentiation of NMDARs as a possible mechanism in mediating VTA long-term plasticity produced by cocaine.

An increase in NR2B-containing NMDARs mediates longer lasting NMDAR EPSCs, likely making DA cells more responsive to excitatory inputs after cocaine withdrawal. Thus, patterns of excitatory afferent activity in the VTA that were subthreshold for eliciting LTP before cocaine exposure could now elicit LTP. Such a DA-mediated change in NMDAR EPSCs in the VTA may be functionally important, because local changes in DA neurotransmission elicited by injections of cocaine or the DA reuptake inhibitor GBR12909 into the VTA can cause behavioral sensitization to subsequent systemic cocaine injections (Cornish and Kalivas, 2001). Repeated local injections of the D_1/D_5 receptor agonist SKF38393 into the VTA also produce behavioral sensitization to systemic cocaine (Pierce et al., 1996). Previous studies have shown that the D_1/D_5 receptor antagonist SCH23390 blocks context-dependent or one-shot sensitization (Fontana et al., 1993) and the development of sensitization to cocaine as measured at 3 d of withdrawal (White et al., 1998). These studies suggest that sensitization to cocaine may involve DA D_5 receptors in the VTA. Thus, activation of DA D_5 receptors in the VTA may be an important mechanism that generally contributes to the addictive properties of cocaine and may represent a novel target for treating cocaine addiction.

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