Signaling via Dopamine D1 and D3 Receptors Oppositely Regulates Cocaine-Induced Structural Remodeling of Dendrites and Spines

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Cocaine • Dopamine receptors • Signal transduction • NMDA receptor • Extracellular signal-regulated kinase • Dendrite • Myocyte enhancer factor 2 activity

Abstract
Repeated exposure to cocaine can induce persistent alterations in the brain. The structural remodeling of dendrites and dendritic spines is thought to play a critical role in cocaine addiction. We previously demonstrated that signaling via dopamine D1 and D3 receptors have opposite effects on cocaine-induced gene expression. Here, we show that cocaine-induced structural remodeling in the nucleus accumbens (NAc) and caudoputamen (CPu) is mediated by D1 receptors and inhibited by D3 receptors. In addition, chronic exposure to cocaine results in an altered number of asymmetric spine synapses via the actions of both D1 and D3 receptors. The contradictory effects of D1 and D3 receptor signaling on cocaine-induced structural remodeling is associated with NMDA-receptor R1 subunit (NR1) phosphorylation, and is dependent upon the activation of extracellular signal-regulated kinase (ERK). In addition, we found that D1 and D3 receptor signaling has contradictory effects upon the activation of the myocyte enhancer factor 2 (MEF2), which is involved in the dendritic remodeling after cocaine treatment. Together, these data suggest that dopamine D1 and D3 receptors differentially regulate the cocaine-induced structural remodeling of dendrites and spines via mechanisms involving the consecutive actions of NR1 phosphorylation, ERK activation, and MEF2 activity in the NAc and CPu.

Introduction
Drug addiction involves persistent neuroadaptations in the brain [1–5]. For example, chronic exposure to cocaine results in long-lasting increases in the number of dendrites and the density of dendritic spines in neurons located in areas of the brain involved in reward, such as the nucleus accumbens (NAc) and caudoputamen (CPu) [6–11]. These changes in dendritic morphology are thought to play key roles in cocaine-induced behavioral plasticity and addiction [12]. However, the intracellular signaling mechanism that controls this process is poorly understood.

Dopamine receptors, which include the D1 class (D1 and D5) and the D2 class (D2, D3, and D4), play important roles in cocaine-induced changes in the brain [13–
16]. With the use of D1 and D3 receptor mutant mice, we and others have shown that the activation of these receptors has opposite effects upon cocaine-induced locomotor activity and target gene expression [17–19]. Both acute and chronic exposure to drugs of abuse, such as cocaine and morphine, can induce phosphorylation of the NMDA-receptor R1 subunit (NR1) as well as the activation of extracellular signal-regulated kinase (ERK) in the dopaminergic system, effects that may contribute to the development of persistent changes in the brain [20–26]. In addition, the phosphorylation of NR1 and activation of ERK after acute and chronic cocaine treatment have been shown to be differentially regulated by D1 and D3 receptors [19]. Interestingly, a recent study revealed that chronic cocaine-induced dendritic spine formation is most stable in D1 receptor-expressing neurons in the NAc, indicating that D1 receptor signaling plays an important role in the long-lasting dendritic changes induced by repeated cocaine exposure [27]. A separate study found that cocaine suppresses activity of the myocyte enhancer factor 2 (MEF2) protein via the phosphorylation of MEF2 at Ser408/444 in the NAc, and this reduced activity of MEF2 contributes to the structural remodeling of dendritic spines and the sensitized responses to cocaine [28]. Importantly, this study found that D1 receptor signaling regulates MEF2 activity following cocaine exposure [28].

Although dopamine receptors have been implicated in the mechanisms underlying cocaine-induced dendritic spine formation [27, 28], it remains unclear whether the intracellular signaling pathways downstream of both D1 and D3 receptor activation regulate the cocaine-induced structural remodeling of dendrites and spines. In the current study, we present evidence that D1 and D3 receptor signaling have opposite effects upon the cocaine-induced structural remodeling of dendrites and spines, and that these effects are likely mediated via the consecutive events of NR1 phosphorylation, ERK activation, and MEF2 activity in the NAc and CPu.

**Materials and Methods**

**Mice**

Kunming strain mice, 7–10 weeks of age (mean age 8 weeks), obtained from the Southern Medical University Animals Center (Guangzhou, China) were group-housed in an animal-housing room under a 12-hour light/dark cycle with food and water available ad libitum. The Kunming mouse strain originated from Swiss mice brought to Kunming, China, from the Indian Haffkine Institute in 1944 [29]. Similar numbers of male and female mice were used in the current study. All experimental procedures were in compliance with the National Institutes of Health guidelines and were approved by the local Animal Care and Use Committee of Southern Medical University.

**Drugs**

Cocaine hydrochloride (Qinghai Pharmaceutical Factory, China), the D1 receptor antagonist SCH23390 (Tocris Cookson, Ballwin, Mo., USA), and the NMDA receptor antagonist MK801 (Sigma, St. Louis, Mo., USA) were dissolved in normal saline (hereinafter referred to as saline) [19, 30]. The MEK inhibitor SL327 (Sigma) was dissolved in dimethylsulfoxide and then diluted in saline [18, 31]. The D3 receptor antagonist NGB2904 was dissolved in PEG400 and then diluted in saline [32]. Saline was used as the vehicle (0 dose) control. All injections were administered intraperitoneally (i.p.) in volumes of 10 ml/kg. Injections were performed during the light phase of the light/dark cycle.

**Drug Treatment**

For Western blotting and immunohistochemical analyses, mice (n = 4–6) were injected with cocaine (20 mg/kg) or saline once a day for 7 days, and then analyzed 20 min, 4, 12, 24, 48, or 72 h following the last injection. For Golgi-Cox staining and electron microscopic analysis, mice (n = 4–8) were injected once daily with cocaine (20 mg/kg) or saline 5 days/week for 4 weeks, sacrificed 24 h following the last injection, and then analyzed for the dendritic morphology. The D1 receptor antagonist SCH23390 (0.5 mg/kg), the D3 receptor antagonist NGB2904 (1 mg/kg), and the NMDA receptor antagonist MK801 (0.1 mg/kg) were injected 30 min prior to cocaine administration, while the MEK antagonist SL327 (30 mg/kg) was injected 15 min prior to cocaine administration. It is important to note that saline injections, and some cocaine injections, were preceded by saline injections to control for injection stress. Comparable numbers of male and female mice were used in each group. Our drug treatment time points were based on previous findings that the gene expression pattern induced by 7 consecutive days of cocaine injections is similar to that induced by 28 days of cocaine injections, and that the time course for these changes in gene expression correlates with that for dendritic remodeling [28, 33].

For the pretreatment of animals with SCH23390, MK801, and SL327, doses were based on previous studies in which SCH23390 blocked cocaine target gene expression in the CPu, SL327 decreased cocaine-induced ERK activation and downstream target gene expression in the NAc and CPu, and MK801 decreased amphetamine-induced behavioral sensitization and cocaine-induced ERK activation in the CPu [18, 19, 30, 31, 34]. The dose of NGB2904 pretreatment used is in accordance with previous studies in which NGB2904 enhanced cocaine-induced increases in NAc dopamine concentration and increased amphetamine-stimulated locomotion [32, 35, 36]. In addition, we performed a dose-effect experiment by administering different dosages of NGB2904 (0.1, 0.5, 1, 3, and 5 mg/kg i.p.) before each cocaine treatment to confirm the selectivity of NGB2904 for D3 receptors.

**Dendritic Morphology and Data Analysis**

Twenty-four hours following the final injection, the brains were removed and processed for Golgi-Cox impregnation. Brains were cut into 150-μm sections and medium spiny neurons and basilar dendrites of layer III somatosensory cortex pyramidal neu-
rons were analyzed. The somatosensory cortex was used as a control brain region because it does not have a significant dopaminergic input [37, 38]. Dendritic morphological analysis was carried out in both hemispheres of the NAc, CPu, and somatosensory cortex using three methods [33, 39–41]. First, the dendritic complexity was calculated using a Sholl analysis of ring intersections [33, 39]. Second, the total number of dendritic branches was counted at each branch point from the cell body. Third, spine density was quantified by counting spines on the third-order (or greater) dendritic terminal tip of each medium spiny neuron and on the terminal tip of basilar dendrites from each layer III somatosensory cortex pyramidal neuron. Spines were counted from the last branch point to the terminal tip of the dendrite. Dendritic branches were counted from 8–12 neurons of each mouse in the NAc, CPu, and somatosensory cortex. Dendritic spine density was counted at 1,000× (at least 20 μm in length) from different neurons in the NAc, CPu, and somatosensory cortex of each mouse [33, 39]. All neurons were reconstructed using Image Pro Plus version 5.1 (Media Cybernetics, Silver Spring, Md., USA), which allows three-dimensional analysis of dendritic trees. All measurements were made by a person blinded to experimental condition. Dendrites and spines were compared using one-way ANOVA followed by Bonferroni post-hoc test. Significance was set at p < 0.05.

Electron Microscopy
Twenty-four hours following the last injection, mice (n = 8) were briefly anesthetized with methoxyflurane and cardiac-perfused with 0.9% saline followed by freshly prepared 4% paraformaldehyde with 0.2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. The brains were rapidly removed and stored in the same fixative containing 2.5% glutaraldehyde overnight at 4 °C. Coronal sections (30 μm) containing NAc and CPu were cut using a freezing microtome. One square millimeter blocks were excised from the NAc and CPu at the level of the anterior commissure. Blocks were treated with 1% osmium for 1 h on ice, counterstained with 2% aqueous uranyl acetate for 1 h, dehydrated through an ascending series of ethanols, rinsed in propylene oxide, and flat-embedded in Epon. The flat-embedded specimens were sectioned with a 200-mesh formvar-coated grids. Grids were stained with uranyl acetate for 1 h, dehydrated through an ascending series of ethanols, rinsed in propylene oxide, and flat-embedded in Epon. The flat-embedded specimens were sectioned at 70 nm with an ultramicrotome and mounted on 200-mesh formvar-coated grids. Grids were stained with uranyl acetate followed by lead citrate, and examined with a transmission electron microscope (Hitachi H-7500, Japan).

Synapse counts were conducted in single ultrathin sections from the NAc and CPu, and the number of synapses was assessed semiquantitatively. A microscopist blinded to experimental condition viewed 6 photomicrographs at 12,000× from the NAc and CPu of each mouse. A virtual 15 × 11.25-μm box was placed in each image. The number of asymmetric synapses located within each box was counted. Synapses were identified by the presence of pre- and postsynaptic membrane specializations, a visible synaptic cleft, and the accumulation of synaptic vesicles in the presynaptic profile [42, 43]. Results were analyzed using one-way ANOVA followed by Bonferroni post-hoc test. Significance was set at p < 0.05.

Western Blotting and Data Analysis
The NAc and CPu regions were isolated by gross dissection and protein extracts were prepared as described [31]. Samples were homogenized in 300 μl buffer containing 1% NP-40, 50 mM Tris–HCl (pH 7.5), 50 mM NaCl, 5 mM EDTA, 10 mM EGTA, 2 mM sodium pyrophosphate, 4 mM paranitrophenylphosphate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin and 2 μg/ml pepstatin. Homogenates were incubated on ice and centrifuged at 12,000 g at 4°C. Protein concentrations were determined using the Bradford method. Twenty micrograms of total protein were separated by 10% SDS-PAGE for ERK and NR1 immunoblotting as described [18, 31]. The resolved proteins were transferred onto PVDF membranes, and the blots were blocked in 5% nonfat dry milk, 10 mM Tris–HCl (pH 7.5), and 0.1% Tween 20 prior to incubation in primary antibodies followed by horseradish peroxidase (HRP)-conjugated secondary antibodies. Antibodies against ERK, phospho-ERK, phospho-NR1 (Cell Signaling Technology, Beverly, Mass., USA) and NR1 (Upstate Inc., Lake Placid, N.Y., USA) were used at a 1:1,000 dilution, antibodies against actin (Santa Cruz) were used at a 1:3,000 dilution. The HRP-anti-rabbit conjugated secondary antibody (Santa Cruz) was used at a 1:5,000 dilution on the ERK and NR1 immunoblots. To normalize, the immunoreactive intensity of phospho-ERK and phospho-NR1 bands was divided by the intensity of total ERK and total NR1 bands, respectively. All Western blot analyses were performed at least three times, and parallel results were obtained. Statistical analysis was performed using one-way ANOVA followed by Bonferroni post-hoc test. Significance was set at p < 0.05.

Immunohistochemistry
For the phospho-MEF2A (Ser408) immunostaining, 4–6 mice in each group were anesthetized and perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. Brains were removed and post-fixed in 4% paraformaldehyde for 2 h, then were cryoprotected in 20% sucrose at 4 °C overnight. Freshly frozen coronal sections (30 μm) were cut using a cryostat. Free-floating sections were blocked with 0.4% Triton-100 and 0.1% BSA in PBS for 1 h and were incubated at 4 °C overnight in PBS, 0.4% Triton-100 and 0.1% BSA containing a phosphorylation site-specific antibody to P-Ser408 MEF2A (Cell Signaling) at 1:100 dilution. Sections were then incubated for 1 h at room temperature with a biotin-conjugated secondary antibody, followed a 1-hour incubation in an avidin-biotin-peroxidase complex (ABC) solution (Vector Laboratories). The sections were then developed using the diaminobenzidine (DAB) reaction. These experiments were repeated three times using multiple brain sections. Quantification of immunoreactive nuclei in DAB-stained sections from the NAc and CPu was performed on images captured using a light microscope with a 20× objective. Image Pro Plus software was used to count intensely labeled nuclei for each digitized image covering an area of 10 mm² with a 5× background threshold. Data were expressed as total immunoreactive nuclei per square millimeter, or the ratio of MEF2-positive cells in cocaine- to saline-treated mice. Statistical analysis was performed using one-way ANOVA followed by Bonferroni post-hoc test. Significance was set at p < 0.05.

Immunohistochemistry for phospho-MEF2A (Ser408) and dynorphin was performed as follows. Mice (n = 4) were perfused 24 h after the last injection following 7 consecutive days of cocaine treatments. We performed two sets of controls to confirm the specificity of double immunolabeling. First, the omission of the primary antibodies resulted in no immunoreactive signal, suggesting that the secondary antibodies lack nonspecific binding to sections under the conditions used. Second, the omission of one of the two primary antibodies abolished immunoreactivity for the omitted antibody without affecting that of the other, indicating

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that the secondary antibody did not show any significant cross-reactivity, and labeling sections with one antibody did not interfere with the second primary antibody. Brain sections were then incubated overnight at 4°C in a cocktail of two primary antibodies: a phospho-specific rabbit polyclonal antibody directed against the P-Ser408 epitope of MEF2A (Cell Signaling, 1:100 dilution) and a guinea pig polyclonal antibody that recognizes prodynorphin (Abcam, 1:100 dilution) [18]. Sections were then incubated with Alexa Fluor 488-conjugated and Alexa Fluor 546-conjugated (Molecular Probes, 1:250 dilution) secondary antibodies to visualize cells immunoreactive for both phospho-MEF2A (Ser408) and dynorphin. Images of each section were captured by Image Pro Plus program. This experiment was repeated at least three times using multiple brain sections.

Postnatal Prefrontal Cortex Cultures and Data Analysis

Postnatal prefrontal cortex (PFC) neurons were isolated as described previously [44, 45]. Briefly, postnatal day-1 Sprague-Dawley rats were decapitated and the PFC was rapidly dissected. Tissue was dissociated by incubation in 0.125% trypsin at 37°C for 10 min. Cells were plated onto coverslips coated with poly-D-lysine (100 µg/ml, Sigma) in 24-well culture plates at a density of 20,000 cells/plate. Cells were grown in Neurobasal media (Invitrogen, Gaithersburg, Md., USA) supplemented with 2% B27 (Invitrogen) and 0.5 mM glutamine with 0.5 µM cytosine arabinoside (AraC) added at day in vitro (DIV) 2.

PFC neurons were treated with PBS or the D1-receptor agonist SKF81297 (1 µM, 30 min) on DIV 11, 13, and 15. Neurons were pretreated with the NMDA receptor antagonist MK801 (20 µM) for 15 min before SKF81297 treatment. Four days following intermittent treatment, the structural remodeling of dendrites and spines on the cultured neurons was assessed. Coverslips were incubated with a mouse monoclonal antibody against MAP2 (1:1,000; Chemicon International, Temecula, Calif., USA) to visualize dendrites, or a polyclonal sheep antibody against γ-actin (1:1,000; Chemicon) to visualize dendritic spines. Primary antibodies were detected using anti-mouse Alexa Fluor 546- or anti-sheep Alexa Fluor 488-conjugated secondary antibodies (1:600; Molecular Probes Invitrogen, Carlsbad, Calif., USA). In the current study, we defined dendritic spines to be round or mushroom-shaped, and <3 µm long [46]. For each experimental group, cells from at least 3 different coverslips were used and approximately 8 cells from each coverslip were analyzed. All images were acquired using Image Pro Plus version 5.1 (Media Cybernetics, Silver Spring, Md., USA). Data from the different groups were compared using one-way ANOVA followed by Bonferroni post-hoc test. Significance was p < 0.05.

Results

Cocaine-Induced Structural Remodeling of Dendrites and Spines Is Promoted by D1 Receptor and Inhibited by D3 Receptor Activation

We and others have previously found that the dopamine D1 and D3 receptors make opposing contributions to cocaine-induced locomotor activity and cocaine-induced gene expression [17–19, 31, 47]. To determine the role of dopamine receptors in the cocaine-induced structural remodeling of dendrites and dendritic spines in the NAc and CPu, we used a well-established cocaine treatment protocol to induce dendritic spines in the NAc [41]. Mice were treated with cocaine or saline 5 days/week for 4 weeks, and dendritic morphological analyses were conducted. The D1 receptor inhibitor SCH23390 and the D3 receptor antagonist NGB2904 were administered 30 min prior to cocaine or saline injections, respectively. The treatment paradigm is indicated below the images (fig. 1F). Measurements were made by 2 investigators blind to experimental conditions. As shown in figure 1A–D, repeated cocaine injections led to an increase in dendritic branching (22.2% more in NAc: 23.44 ± 0.643 vs. 19.17 ± 0.624, p < 0.001, n = 48 neurons; 23.3% more in CPu: 30.88 ± 0.908 vs. 25.04 ± 1.036, p < 0.001, n = 48 neurons) and spine density (28.1% more in NAc: 12.3 ± 0.38 vs. 9.6 ± 0.27, p < 0.001, n = 45 dendrites; 29.4% more in CPu: 12.4 ± 0.42 vs. 9.58 ± 0.31, p < 0.001, n = 45 dendrites) of the medial spiny neurons.

Interestingly, pretreatment of mice with NGB2904 increased the number of dendrites by 11.1% in the NAc (26.04 ± 0.756 vs. 23.44 ± 0.643, p = 0.034, n = 48 neurons) and by 11.8% in the CPu (34.52 ± 0.992 vs. 30.88 ± 0.908, p = 0.021, n = 48 neurons). NGB2904 pretreatment also increased spine density by 13.8% in the NAc (14.8 ± 0.38 vs. 12.3 ± 0.38, p < 0.001, n = 45 dendrites) and by 12.1% in the CPu (13.87 ± 0.43 vs. 12.4 ± 0.42, p = 0.001, n = 48 neurons). Pretreatment with the D1 receptor antagonist SCH23390 (0.5 mg/kg), the D3 receptor antagonist NGB2904 (1 mg/kg) and saline were administered 30 min before cocaine or saline administration. A Representative dendritic branching images of MSNs for the NAc and CPu. Scale bar 10 µm. B Representative dendritic spine images of MSNs for the NAc and CPu. Scale bar 10 µm. C, D Quantification of dendritic branching (C) and dendritic spine density (D). Data represent mean ± SEM; n = 4–6 mice. * p < 0.05 compared with saline-treated mice; ** p < 0.05 compared with cocaine-treated mice (for dendritic branching, F(3,188) = 12.929 for NAc and F(3,188) = 33.047 for CPu; for spine density, F(3,176) = 38.609 for NAc and F(3,176) = 30.176 for CPu). E The changes in dendrite complexity in the NAc and CPu revealed by Sholl analysis of the intersection number per 20 µm radial unit distance from soma of MSNs. n = 4–6 mice. * p < 0.05 compared with saline-treated mice; ** p < 0.05 compared with cocaine-treated mice. F Cocaine treatment regimens: Sal/Sal = Saline/saline; Sal/Coc = saline/cocaine; SCH/Coc = SCH23390/cocaine; NGB/Coc = NGB2904/cocaine.
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0.04, n = 45 dendrites) of MSNs. In contrast, pretreatment of mice with SCH23390 inhibited the cocaine-induced increase in the number of dendrites (22.6% fewer in the NAc, 18.13 ± 0.601 vs. 23.44 ± 0.643, p < 0.001, n = 48 neurons; 22.7% fewer in the CPU, 23.88 ± 0.39 vs. 30.88 ± 0.908, p < 0.001, n = 48 neurons) and spine density (20.3% fewer in NAc, 9.8 ± 0.26 vs. 12.3 ± 0.38, p < 0.001, n = 45 dendrites; 22.0% fewer in CPU, 9.67 ± 0.37 vs. 12.4 ± 0.42, p < 0.001, n = 45 dendrites) of MSNs.

As a complementary measurement of dendritic complexity, we performed a standard Sholl analysis, which counts the number of dendritic intersections with concentric circles centering on the cell soma and spaced 20 μm apart. As shown in figure 1E, we observed an increased dendritic complexity following repeated cocaine treatment compared to saline-treated mice, represented by an increased number of dendritic intersections in the NAc and CPU from cocaine-treated mice. Pretreatment of mice with SCH23390 reduced the number of dendritic intersections in the NAc and CPU (fig. 1E). In contrast, pretreatment of mice with NGB2904 increased the number of dendritic intersections in the NAc and CPU. Numerical data from the Sholl analyses are presented in online supplementary table S1-1 (for all online suppl. materials, see www.karger.com/doi/10.1159/000330743). The number of dendrites and the spine densities on medium spiny neurons in the NAc and CPU were similar between the mice treated with SCH23390 or NGB2904 alone compared with saline-treated mice (online suppl. fig. S1). In addition, the observed alterations in the NAc and CPU following repeated cocaine treatment appear to be specific, as no obvious effects of cocaine were observed on the dendritic branching and spine density in the basilar dendrites of layer III somatosensory cortex pyramidal neurons (online suppl. fig. S2).

NGB2904 has been reported to have a greater than 150-fold selectivity for primate D3 over D2 receptors, and an 800-fold selectivity for rat D3 versus D2 receptors [48, 49]. A number of in vivo studies further support the selectivity of NGB2904 for D3 receptors [32, 35, 36]. To confirm the selectivity of NGB2904 in our experiments, we conducted a dose-response experiment by treating mice with different dosages of NGB2904 (0.1, 0.5, 1, 3, and 5 mg/kg, i.p.) prior to each cocaine treatment. We found that there was a significant difference between the different NGB2904 treatment groups on measures of dendritic branching (CPU, F(5,282) = 12.779, p < 0.001; NAc, F(5,282) = 13.806, p < 0.001) and spine density (CPU, F(5,264) = 11.911, p < 0.001; NAc, F(5,264) = 13.107, p < 0.001; online suppl. table S2). Although we observed a trend towards an increased effect on dendritic remodeling with escalating NGB2904 doses, only dosages in the range of 1–5 mg/kg (i.p.) significantly increased cocaine-induced dendritic branching and spine density (online suppl. fig. S3). Individual group comparisons using Bonferroni post-hoc tests revealed statistically significant differences between the 0.1- and 1-mg/kg NGB2904 groups (dendritic branching in CPU, 30.71 ± 0.735 vs. 34.1 ± 0.823, p = 0.041; dendritic branching in NAc, 23.52 ± 0.619 vs. 26.19 ± 0.576, p = 0.024; spine density in CPU, 12.78 ± 0.19 vs. 13.93 ± 0.325, p = 0.043; spine density in NAc, 12.51 ± 0.359 vs. 13.96 ± 0.313, p = 0.029) and between the 1- and 5-mg/kg groups (dendritic branching in CPU, 34.1 ± 0.823 vs. 37.6 ± 0.674, p = 0.03; dendritic branching in NAc, 26.19 ± 0.576 vs. 28.9 ± 0.607, p = 0.025; spine density in CPU, 13.93 ± 0.325 vs. 15.09 ± 0.318, p = 0.044; spine density in NAc, 13.96 ± 0.313 vs. 15.33 ± 0.242, p = 0.045; online suppl. table S2). Together, these results indicate that the D1 receptor activity positively regulates cocaine-induced structural remodeling of dendrites and dendritic spines in the NAc and CPU, while D3 receptors negatively regulate cocaine-induced remodeling.

**Chronic Exposure to Cocaine Results in Altered Numbers of Asymmetric Spine Synapses Mediated via the Dopamine D1 and D3 Receptors**

A recent report demonstrated that prenatal exposure to cocaine is associated with an increased number of asymmetric spine synapses [50]. To determine cocaine-induced changes in striatal dendrites in more detail, we evaluated the number of asymmetric spine synapses in the NAc and CPU following repeated cocaine treatment using transmission electron microscopy (EM). As shown in figure 2, the estimated number of excitatory asymmetric synapses was significantly increased in both the NAc and CPU following repeated exposure to cocaine (28.8% more in NAc: 20.1 ± 0.308 vs. 15.6 ± 0.25, p < 0.001, n = 48 images; 25.2% more in CPU: 20.38 ± 0.296 vs. 16.27 ± 0.243, p < 0.001, n = 48 images).

To further determine whether the observed increases in asymmetric spine synapse number depends upon dopamine receptor signaling, we treated mice with either SCH23390 or NGB2904 prior to cocaine treatment. We found that the number of asymmetric spine synapses was enhanced in the NAc and CPU of NGB2904-pretreated mice (12.9% more in NAc: 22.69 ± 0.418 vs. 20.1 ± 0.308, p = 0.002, n = 48 images; 11.1% more in CPU: 22.65 ± 0.324 vs. 20.38 ± 0.296, p = 0.008, n = 48 images) and reduced in the SCH23390-pretreated mice (23.2% fewer
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The Differential Effects of D1 and D3 Receptor Activity upon Cocaine-Induced Structural Remodeling Are Associated with the Phosphorylation of NR1

NMDA receptors play key roles in the activity-dependent structural remodeling of dendritic arbors and spines [51–54]. Importantly, NMDA receptors have been implicated in cocaine-induced neuroadaptation [55–57]. Our
previous work suggests that D1 and D3 receptors differentially modulate the NMDA-induced phosphorylation of NR1, specifically at Ser897 [19]. In addition, Ser897 phosphorylation is necessary for D1 receptor-mediated gene expression [24]. Therefore, we speculated that D1 and D3 receptor activity might mediate cocaine-induced structural remodeling of dendrites and spines by influencing NR1 activity at Ser897. To test this possibility, we first determined the effects of D1 and D3 receptor activity upon cocaine-induced phosphorylation of NR1 in the striatum. We treated mice with SCH23390, NGB2904, or MK801 30 min prior to each cocaine injection. We then prepared protein extracts from the CPu and NAc 20 min following the final injection and performed Western blot analyses for NR1 activation using an anti-phospho-NR1 (Ser897) antibody. As shown in figure 3, chronic cocaine treatment induced significant Ser897 phosphorylation 20 min following the final cocaine injection in the NAc (fig. 3A) and CPu (fig. 3B). Ser897 phosphorylation was enhanced in the NAc and CPu in NGB2904-pretreated mice, and inhibited in SCH23390-pretreated mice. Similarly, cocaine-induced Ser897 phosphorylation was inhibited in MK801-pretreated mice. These results suggest that the chronic cocaine-induced phosphorylation of NR1 at Ser897 in the NAc and CPu is differentially regulated by D1 and D3 receptor activity.

We then sought to determine whether NMDA receptors contribute to the increased number of dendrites and dendritic spines observed following chronic cocaine treatment. We treated mice with MK801, 5 days/week for 4 weeks, prior to cocaine or saline injection, and then performed morphological analyses. We found that pretreatment of mice with MK801 prevented cocaine-induced increases in the number of dendrites (22.0% fewer in NAc: 18.29 ± 0.648 vs. 23.44 ± 0.643, p < 0.001, n = 48 neurons; 25.9% fewer in CPu: 25.88 ± 0.908, p < 0.001, n = 48 neurons; fig. 4A, C) and spine density (18.3% fewer in NAc: 12.3 ± 0.38, p < 0.001, n = 45 dendrites; fig. 4B, D). Sholl analysis indicated that the dendrite branching pattern was reduced in MK801 pretreated, compared to not pretreated, cocaine-treated mice in the NAc and CPu (fig. 4E; online suppl. table S1-2). The number of dendrites and spine density of MSNs in the NAc and CPu were similar in mice treated with MK801 alone compared
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**Fig. 4.** Inhibition of NMDA receptors inhibits the structural remodeling of dendrites and spines of MSNs in the NAc and CPu after repeated cocaine treatment. Mice were treated with cocaine (20 mg/kg dose) or saline once daily for 4 weeks and then the dendritic morphological analyses were performed. The NMDA receptor inhibitor MK801 (0.1 mg/kg) and saline were administered 30 min before cocaine or saline injections. **A** Representative dendritic branching images of MSNs for the NAc and CPu. Scale bar 10 μm. **B** Representative dendritic spine images of MSNs for the NAc and CPu. Scale bar 10 μm. **C, D** Quantification of dendritic branching (**C**) and dendritic spine density (**D**) in the indicated groups of mice. Data represent mean ± SEM. *p < 0.05 compared with saline-treated mice; **p < 0.05 compared with cocaine-treated mice (for dendritic branching, F(2,141) = 18.60 for NAc and F(2,141) = 12.929 for CPu; for spine density, F(2,132) = 20.113 for NAc and F(2,132) = 16.795 for CPu). **E** The changes in dendrite complexity in the NAc and CPu revealed by Sholl analysis of the intersection number per 20 μm radial unit distance from soma of MSNs. Statistical analysis was performed using one-way ANOVA followed by Bonferroni post-hoc test. Data represent mean ± SEM. *p < 0.05 compared with cocaine-treated mice.
Thus, NMDA receptor activity is required for the cocaine-induced structural remodeling of dendrites and dendritic spines in the NAc and CPu.

To determine whether D1 receptors act via NMDA receptors to mediate cocaine-induced remodeling, we tested whether MK801 would block the dendritic changes induced by a D1 receptor agonist in cultured PFC neurons. PFC neurons were treated with PBS or the D1-like agonist SKF81297 on DIV 11, 13, and 15. Four days after treatment, dendrites and dendritic spines were analyzed. As shown in figure 5, SKF81297 treatment led to an increase in dendritic branching (14.83 ± 0.51 vs. 24.75 ± 0.41, p < 0.001, n = 24 neurons) and spine density (7.68 ± 0.36 vs. 9.043 ± 0.27, p < 0.001, n = 24 dendrites) in cultured PFC neurons compared to the PBS-treated group. Importantly, MK801 pretreatment inhibited the SKF81297-induced increase in dendritic branching (14.41 ± 0.43 vs. 24.75 ± 0.41, p < 0.001, n = 24 neurons) and spine density (7.07 ± 0.48 vs. 9.043 ± 0.27, p < 0.001, n = 24 dendrites), suggesting that the effects of D1 receptor activation on dendritic remodeling may be mediated via NMDA receptor signaling. These results indicate that the opposing effects of D1 and D3 receptor signaling upon cocaine-induced structural remodeling may require the activity of NMDA receptors.

D1 and D3 Receptor Signaling-Mediated ERK Activation Contributes to the Cocaine-Induced Structural Remodeling of Dendrites and Dendritic Spines

As cocaine exposure induces ERK activation in the striatum [21], and the regulation of cocaine-induced gene expression by both dopamine receptors and NMDA receptors depends upon ERK activation [18, 19, 25], we hypothesized that ERK activation may be required for the differential effects of D1 and D3 receptor signaling upon cocaine-induced structural remodeling in the striatum. To test this hypothesis, we treated mice once daily for 7 days with the D1 receptor inhibitor SCH23390, the D3

Fig. 5. Inhibition of NMDA receptors suppresses the structural remodeling of dendrites and spines of PFC neurons induced by D1 receptor agonist treatment. PFC neurons were treated with either PBS or the D1-like agonist SKF81297 (1 μM, 30 min) on days 11, 13 and 15 in culture. The NMDA receptor antagonist MK801 (20 μM) were pre-incubated 15 min before SKF81297 treatment. Four days after intermittent treatment, cells were analyzed for the structural remodeling of dendrites and spines.
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Fig. 6. The activation of ERK after repeated cocaine treatment is oppositely regulated by D1 and D3 receptors and is also NMDA receptor-dependent. Mice (n = 4–6) were treated with cocaine (20 mg/kg) or saline once a day for 7 days. The D1 receptor inhibitor SCH23390 (0.5 mg/kg), the D3 receptor antagonist NGB2904 (1 mg/kg), the NMDA receptor inhibitor MK801 (0.1 mg/kg) and saline were administered 30 min before cocaine or saline injections. The NAc (A) and CPu (B) protein extracts were prepared 20 min after the last injection. Western blot analyses were performed using antibody against dually phosphorylated (Thr202 and Tyr204) ERK (pERK) or total ERK. Data represent the mean ± SEM. Saline-treated levels were set at 1 for quantifications. * p < 0.05 compared with saline-treated mice; ** p < 0.05 compared with cocaine-treated mice (for NAc, F(4,21) = 282.525, for CPu, F(4,21) = 225.475).

To determine whether ERK activation contributes to cocaine-induced structural remodeling in the striatum, we injected mice with the ERK inhibitor SL327 daily for 4 weeks 30 min prior to cocaine or saline injection. We then performed morphological analyses and found that the pretreatment of mice with SL327 inhibited the cocaine-induced increases in dendrite number (17.5% fewer in NAc: 19.33 ± 0.621 vs. 23.44 ± 0.643, p < 0.001, n = 48 neurons; 18.7% fewer in CPu: 25.1 ± 0.85 vs. 30.88 ± 0.908, p < 0.001, n = 48 neurons; fig. 7A, C) and spine density (12.2% fewer in NAc: 10.8 ± 0.33 vs. 12.3 ± 0.38, p < 0.001, n = 45 dendrites; 14.5% fewer in CPu: 10.62 ± 0.354 vs. 12.4 ± 0.42, p < 0.001, n = 45 dendrites; fig. 7B, D). Sholl analyses indicated that the dendrite-branching pattern was simplified in SL327 pretreated mice compared with control cocaine-treated mice in the NAc and CPu (fig. 7E; online suppl. table S1-3). The number of dendrites and the density of dendritic spines on medium spiny neurons in the NAc and CPu were similar between the SL327 alone-treated and saline-treated mice (online suppl. fig. S1). These data suggest that ERK activation, differentially regulated by D1 and D3 receptor activity, is required for cocaine-induced structural remodeling in the NAc and CPu.
Fig. 7. Inhibition of ERK activity prevents the structural remodeling of dendrites and spines of MSNs in the NAc and CPu after repeated cocaine treatment. Mice (n = 4–6) were treated with cocaine (20 mg/kg) or saline once a day for 4 weeks and then the dendritic morphological analyses were performed. The MEK antagonist SL327 (30 mg/kg dose) and saline were administered 15 min before cocaine or saline injections. A Representative dendritic branching images of MSNs for the NAc and CPu. Scale bar 10 μm. B Representative dendritic spine images of MSNs for the NAc and CPu. Scale bar 10 μm. C, D Quantification of dendritic branching (C) and dendritic spine density (D) in the indicated groups of mice. Data represent mean ± SEM. * p < 0.05 compared with saline-treated mice; ** p < 0.05 compared with cocaine-treated mice (for dendritic branching, F(2,141) = 14.761 for NAc and F(2,141) = 12.848 for CPu; for spine density, F(2,132) = 16.313 for NAc and F(2,132) = 15.380 for CPu). E The changes in dendrite complexity in the NAc and CPu revealed by Sholl analysis of the intersection number per 20 μm radial unit distance from soma of MSNs. * p < 0.05 compared with cocaine-treated mice.
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**Fig. 8.** Time course (A) and quantification (B) of cocaine-induced MEF2 phosphorylation at Ser408 in the NAc and CPu after repeated cocaine exposure. Mice (n = 4–6) were treated with cocaine (20 mg/kg) or saline once daily for 7 consecutive days. Coronal sections were stained with a phosphorylation site-specific antibody to P-Ser408 MEF2A. P-MEF2-immunoreactive cells in the NAc and CPu were counted 4, 12, 24, 48, and 72 h after the last injection. Scale bar 200 μm. Data represent ratio of the number of P-Ser408 MEF2A-positive cells in 1 mm² (mean ± SEM) area in cocaine-treated mice vs. saline-treated mice. Saline-treated levels were set at 1 for quantifications. * p<0.05 compared with saline-treated mice.
D1 and D3 Receptor Activity Differentially Modulates MEF2 Activity following Chronic Cocaine Treatment

Members of the MEF2 family (MEF2A–MEF2D) are highly expressed in the brain [58]. A recent study showed that cocaine exposure suppresses the activity of MEF2 in the NAc via the phosphorylation of MEF2 at Ser408/444, and that the reduced activity of MEF2 contributes to the structural remodeling of dendritic spines in the NAc and sensitized responses to cocaine [28]. Importantly, MEF2 activity is regulated by D1 receptor signaling following cocaine treatment [28]. To determine whether the involvement of MEF2 on structural remodeling in the striatum is regulated by D1 and D3 receptor signaling pathways, we treated mice with the D1 and D3 receptor antagonists prior to chronic cocaine administration. Animals were then analyzed 4, 12, 24, 48, and 72 h following the final injection, and the striatum was isolated for Western blot analysis using an antibody that recognized phospho-Ser408 MEF2A immunoreactivity. * p < 0.05 compared with cocaine-treated mice.
Immunopositive nuclei were defined by an arbitrary threshold of 5× background in digitized images using Image Pro Plus [40]. Similar to previous reports [28], cocaine exposure produced a time-dependent increase in MEF2 phosphorylation at Ser408, with a maximal effect observed 24 h following the last cocaine injection, compared with saline-treated control mice (fig. 8). However, unlike previous reports, the levels of MEF2 phosphorylation returned to baseline 72 h, instead of 48 h, following the final cocaine injection. Importantly, the degree of MEF2 phosphorylation at Ser408 was enhanced in NGB2904-pretreated mice (p < 0.05) and decreased in SCH23390-pretreated mice (p < 0.05) 24 h following the final cocaine injection (fig. 9). To determine whether the phosphorylation of MEF2 at Ser408 depends upon NMDA receptor signaling and ERK activation, we pretreated mice with MK801 and SL327. We found that both MK801 and SL327 inhibited the phosphorylation of MEF2 at Ser408 24 h following the final cocaine injection (fig. 9). Immunohistochemical analyses revealed that the levels of Ser408 phosphorylation in medium spiny neurons in the NAc and CPu were similar in mice treated solely with SCH23390, NGB2904, MK801 or SL327, compared to saline-treated mice (online suppl. fig. S4). Together, these findings suggest that the D1 and D3 receptors differentially regulate MEF2 phosphorylation following chronic cocaine treatment, likely through NMDA receptor activation and ERK signaling.

To determine further whether cocaine-induced MEF2 phosphorylation occurs primarily in D1 receptor-expressing neurons in the striatum, we analyzed the distribution of phospho-MEF2A and dynorphin in the NAc by double-label immunohistochemistry [59]. We evaluated the phosphorylation of MEF2 phosphorylation at the 24-hour time point, when cocaine induces the maximal MEF2A Ser408 phosphorylation (fig. 10B) and dynorphin expression (fig. 10A). As shown in figure 10D, phospho-Ser408 colocalizes with dynorphin in the cytoplasm of cells in the NAc. A subset of phospho-Ser408 immunoreactive neurons did not contain dynorphin immunoreactivity. These results indicate that the phosphorylation of MEF2A at Ser408 occurs primarily in D1 receptor-expressing neurons in the striatum. Together with the regulatory role of MEF2 in chronic cocaine-induced dendritic remodeling, our data suggest that cocaine exposure activates MEF2, which in turn effects rapid dendritic remodeling in the striatum via dopamine receptor-dependent pathways.

Discussion

Dendritic remodeling has been hypothesized to contribute to the long-lasting behavioral sensitization seen following chronic cocaine administration [39, 60]. Likewise, it has recently become clear that the structural plasticity of dendritic spines is associated with synaptic plasticity [61, 62]. We previously demonstrated that the D1 and D3 receptor signaling elicits opposing regulatory effects upon cocaine-induced gene expression [18, 19]. In the current study, we found that D1 and D3 receptor activity differentially regulates the cocaine-induced structural remodeling of dendrites and dendritic spines, likely via a mechanism that involves the consecutive activating phosphorylation of NR1, ERK, and MEF2 in the NAc and CPu.

It has been shown that D1 and D3 receptors mediate both opposing and synergistic responses in the NAc and CPu [17–19, 63, 64]. Our previous studies showed that the D1 and D3 receptors exert opposite regulation on target gene expression by regulating ERK activation and c-Fos induction following cocaine treatment [18, 19]. A recent study indicated that D1 receptor signaling plays an impor-
tant role in the long-lasting dendritic changes induced by cocaine exposure [27]. An additional study revealed that D1 receptor signaling contributes to cocaine-induced increases in dendritic spine density via the inhibition of MEF2 activity [28]. In the current study, we found that pretreatment of mice with a D1 receptor inhibitor impaired the ability of cocaine to increase the number of dendrites and the dendritic spine density of medium spiny neurons in the NAc and CPu following repeated cocaine administration, whereas pretreatment of mice with a D3 receptor inhibitor increased the number of dendrites and spine density in these neurons. These findings indicate that D1 and D3 receptor activities have opposing effects upon chronic cocaine-induced structural remodeling of dendrites and spines in the NAc and CPu.

In the striatum, synapses onto dendritic spines are primarily asymmetric synapses formed by excitatory afferents from the cortex and thalamus [65]. Recent data demonstrated that prenatal exposure to cocaine is associated with increased numbers of asymmetric spine synapses [50]. In most cases, the changes in dendritic structure assessed by Golgi staining are accompanied by changes in the number of synapses analyzed with EM [66–68]. We proposed that the structural changes in medium spiny neurons following cocaine exposure might be associated with an altered number of asymmetric spine synapses in the striatum. Indeed, this turned out to be the case. Importantly, cocaine-induced alterations in the number of asymmetric synapses was inhibited by D1 receptor inhibition, and increased by D3 receptor inhibition, further emphasizing the opposing roles of D1 and D3 receptors in cocaine-induced remodeling.

NMDA receptors have been implicated in cocaine-induced sensitization [57, 69], locomotor hyperactivity [70, 71], gene expression [24, 72], and structural remodeling of dendritic spines [52, 53]. Importantly, dopamine receptor and NMDA receptor activities functionally converge through both direct physical association and the activation of cell signaling pathways [24, 73–77]. One of the molecular connections between dopamine receptor and NMDA receptor activity in striatal neurons is the phosphorylation of NR1 at Ser897 [78], a modification that was recently shown to be increased by NMDA and which is oppositely regulated by D1 and D3 receptor activity [19]. In the current study, we found that chronic cocaine treatment induces significant phosphorylation of NR1 at Ser897 in the NAc and CPu, and this phosphorylation was differentially regulated by D1 and D3 receptor activity following chronic cocaine treatment. The phosphorylation of NR1 at Ser897 represents an event that could initiate a variety of molecular programs underlying long-term neuroadaptation to cocaine. In agreement with this concept, we found that the NMDA receptor inhibitor prevented the structural remodeling of dendrites and spines following repeated cocaine treatment. Additionally, we carried out an in vitro PFC analysis. Pyramidal neurons from PFC and medium spiny neurons share a common synaptic architecture involving dopamine and an excitatory input converging onto a spine [79]. The intact PFC contains many intrinsic glutamate neurons, accordingly primary PFC cultures contain dendritic spines [80, 81]. On the other hand, growth alone in culture, medium spiny neurons from striatum did not express a spontaneous network and were virtually devoid of dendritic spines [82]. Our in vitro analyses showed that MK801 blocked dendritic changes in cultured neurons induced by the D1 receptor agonist, suggesting that D1 receptor signaling may act via NMDA receptor activation. These data suggest that the activity of D1 and D3 receptors mediate phosphorylation of NR1 at Ser897 and further suggest that the D1, D3, and NMDA receptors work cooperatively to facilitate the structural remodeling observed following chronic cocaine treatment.

ERK has been implicated in the rewarding effects of various drugs of abuse. Acute and chronic cocaine administration can activate ERK in different brain regions [20, 21]. ERK activation participates in long-lasting behavioral effects resulting from drug exposure, and is involved in the structural remodeling of dendritic spine synapses [83–85]. Importantly, ERK activation following cocaine treatment is differentially regulated by D1 and D3 receptors [18]. In the current study, we found that, in parallel with NR1 phosphorylation at Ser897, ERK activation was increased by D1 and decreased by D3 receptor activities, and was also mediated by NMDA receptor activity, indicating that the effects of dopamine receptor activation upon the regulation on ERK activation may occur via phosphorylation of NR1 following chronic cocaine treatment. In addition, we found that ERK activation is required for the structural remodeling of dendrites and dendritic spines following repeated cocaine administration in the NAc and CPu. We previously demonstrated that D1 and D3 receptor-mediated ERK activation contributes to target gene expression following repeated cocaine treatment [18]. In extension of these previous findings, our current data indicate that D1 and D3 receptor signaling-mediated ERK activation is required for the cocaine-induced structural remodeling of dendrites and spines.

Members of the MEF2 family are tightly regulated by several distinct signaling pathways, one of which involves
cyclin-dependent kinase 5 (Cdk5). Cdk5 is a serine/threonine kinase that plays critical roles in neuronal migration, differentiation, and synaptogenesis [86–90]. Chronic cocaine exposure increases the levels and activity of Cdk5 in the NAc [91], and inhibition of Cdk5 activity in the NAc blocks cocaine-induced increases in dendritic spine density [41]. Recent work has shown that MEF2 can regulate excitatory synapse morphogenesis [92, 93], and that cocaine exposure regulates MEF2 function in the NAc to mediate increased in synaptic connectivity [28]. Specifically, chronic cocaine exposure increases MEF2 phosphorylation at its inhibitory Cdk5 site, Ser408/444, in the striatum to suppress its transcriptional activity [28]. Importantly, MEF2 activity in striatal neurons is regulated by dopamine D1 receptor signaling [28]. In the current work, in agreement with previous results [28], we found that chronic cocaine exposure produced a time-dependent increase in MEF2 phosphorylation at Ser408, with a maximal effect observed 24 h following the final cocaine injection. However, unlike previous studies, we observed that the levels of phosphorylated Ser408 levels returned to baseline at 72 h, rather than 48 h, following cocaine exposure. This difference may result from the difference in species, mouse versus rat, used in these studies [28]. Interestingly, we found that Ser408 phosphorylation at the 24-hour time point is differentially regulated by the activity of D1 and D3 receptors. In addition, the NMDA receptor inhibitor and MEK inhibitor also inhibit MEF2 activation induced by chronic cocaine administration. Together, these findings suggest that the activity of MEF2 may be oppositely regulated by the D1 and D3 receptors via NR1 phosphorylation and ERK activation following repeated cocaine treatment.

In the current work, we found that cocaine-induced MEF2 phosphorylation at Ser408 is mostly colocalized with dopamine D1 receptor expression in neurons of the striatum. We previously demonstrated that cocaine-induced ERK activation mostly occurred in D1 receptor-expressing neurons, and that D1 and D3 receptors elicit differential regulation on cocaine target gene expression by regulating ERK activation and c-Fos induction [18]. Two of the target genes that are upregulated by c-Fos following 28 days of cocaine treatment are p35, a specific activator of Cdk5, and Cdk5 itself [33]. Although MEF2 is not known to be a direct substrate of ERK, ERK activation may induce p35 and Cdk5 expression via c-Fos and Egr1, and alterations in Cdk5 expression may lead to changes in MEF2 activity [94]. Together, our findings suggest that, during chronic exposure to cocaine, MEF2 activity in D1 receptor-expressing neurons mediates signaling events that lead to structural remodeling of dendrites and dendritic spines.

After our manuscript had been submitted for publication, a similar finding was published [95]. However, there are several notable differences between this study and ours. First, Ren et al. [95] provided evidence that D1 receptors, NMDA receptors, and ERK contribute to dendritic remodeling induced by repeated cocaine treatment. However, the interactions between these molecules following cocaine treatment were not detected. Our study focused on the differential regulation of D1 and D3 receptor signaling on cocaine-induced structural remodeling of dendrites and spines, and found that D1 and D3 receptors likely elicited their regulation by consecutively regulating NR1 phosphorylation, ERK activation, and MEF2 activity in the NAc and CPu. Secondly, we provide the first evidence that MEF2, a key regulator of dendritic remodeling after cocaine treatment, is differentially regulated by the D1 and D3 receptors through a mechanism likely involving NR1 phosphorylation and ERK activation. Thirdly, the current study provides direct evidence from EM studies showing that chronic cocaine exposure induces alterations in the number of asymmetric spine synapses in the NAc and CPu mediated via the activities of the dopamine D1 and D3 receptors.

In summary, these findings support a model in which the D1 and D3 receptors oppositely regulate cocaine-induced structural remodeling of dendrites and spines, likely via the consecutive regulation of phosphorylation of NR1, ERK activation, and MEF2 activity in the NAc and CPu. In addition, chronic exposure to cocaine results in the altered number of asymmetric spine synapses in the striatum, an effect mediated via D1 and D3 receptor activity. These data provide fundamental insights into the signaling pathways controlling the cocaine-induced structural plasticity that has been implicated in the persistence of drug addiction.

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