Sigma-1 receptor mediates cocaine-induced transcriptional regulation by recruiting chromatin-remodeling factors at the nuclear envelope

Shang-Yi A. Tsai[a,1], Jian-Ying Chuang[a,b,1], Meng-Shan Tsai[a], Xiao-fei Wang[c], Zheng-Xiong Xi[c], Jan-Jong Hung[d], Wen-Chang Chang[e], Antonello Bonci[f,g,h], and Tsung-Ping Su[a,2]

[a]Cellular Pathobiology Section, Integrative Neuroscience Research Branch, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, MD 21224; [b]The Ph.D. Program for Neural Regenerative Medicine, Taipei Medical University, Taipei 11031, Taiwan; [c]Molecular Targets and Medication Discovery Branch, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, MD 21224; [d]Institute of Bioinformatics and Biosignal Transduction, National Cheng Kung University, Tainan 70101, Taiwan; [e]Graduate Institute of Medical Science, Taipei Medical University, Taipei 11031, Taiwan; [f]Cellular Neurobiology Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, MD 21224; [g]Department of Neurology, University of California, San Francisco, CA 94608; and [h]Solomon H. Snyder Neuroscience Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved October 19, 2015 (received for review September 25, 2015)

The sigma-1 receptor (Sig-1R) chaperone at the endoplasmic reticulum (ER) plays important roles in cellular regulation. Here we found a new function of Sig-1R, in that it translocates from the ER to the nuclear envelope (NE) to recruit chromatin-remodeling molecules and regulate the gene transcription thereof. Sig-1Rs mainly reside at the ER–mitochondrion interface. However, on stimulation by agonists such as cocaine, Sig-1Rs translocate from ER to the NE, where Sig-1Rs bind NE protein emerin and recruit chromatin-remodeling molecules, including lamin A/C, barrier-to-autointegration factor (BAF), and histone deacetylase (HDAC), to form a complex with the gene repressor specific protein 3 (Sp3). Knockdown of Sig-1Rs attenuates the complex formation. Cocaine was found to suppress the gene expression of monoamine oxidase B (MAOB) in the brain of wild-type but not Sig-1R knockout mouse. A single dose of cocaine (20 mg/kg) in rats suppresses the level of MAOB at nuclear accumbens without affecting the level of dopamine transporter. Daily injections of cocaine in rats caused behavioral sensitization. Withdrawal from cocaine in cocaine-sensitized rats induced an apparent time-dependent rebound of the MAOB protein level to about 200% over control on day 14 after withdrawal. Treatment of cocaine-withdrawn rats with the MAOB inhibitor deprenyl completely alleviated the behavioral sensitization to cocaine. Our results demonstrate a role of Sig-1R in transcriptional regulation and suggest cocaine may work through this newly discovered genomic action to achieve its addictive action. Results also suggest the MAOB inhibitor deprenyl as a therapeutic agent to block certain actions of cocaine during withdrawal.

sigma-1 receptor | cocaine | MAOB | emerin | deprenyl

The endoplasmic reticulum (ER) plays important roles in cellular functions, including synthesis of proteins and regulation of Ca^{2+} signaling between the ER and plasma membrane (1) and between the ER and mitochondria (2–4). However, because the ER interacts with other organelles in the cell, other functions related to the ER remain to be uncovered.

The sigma-1 receptor (Sig-1R) (5–8) is an ER chaperone molecule that resides at the ER–mitochondrion interface referred to as the mitochondria-associated ER membrane (MAM), where the Sig-1R ensures proper ER–mitochondrion Ca^{2+} signaling for cellular survival (3, 9), as well as sustains the activity of an ER stress sensor IRE1 at the MAM (10). The Sig-1R can translocate from the MAM to plasma membrane of the cell to regulate ion channels and receptors on the plasma membrane (8, 11–14). Cocaine is a Sig-1R agonist (3) that causes the dissociation of Sig-1R from its cognate binding partner BiP (3, 8), and consequently the translocation of Sig-1Rs to the plasma membrane, where Sig-1Rs interact with voltage-gated potassium channel subfamily A member 2 (Kv1.2) to shape the neuronal and behavioral responses to cocaine (15). Cocaine also causes the translocation of Sig-1Rs from the ER to the nucleus (16), where Sig-1Rs are shown to be present at the nuclear envelope (NE) (17). However, the functional role of Sig-1Rs at the nucleus remains unknown.

Cocaine addiction involves genomic alterations that relate to long-term changes in neuronal plasticity (18–22). However, the exact molecular mechanisms that lead to cocaine's genomic alterations remain unclarified. It was reported that cocaine affects the activity of histone deacetylase (HDAC) in the brain, and that the manipulation of HDAC activity influences the action of cocaine (e.g., refs. 21 and 22). However, the exact molecular event relating cocaine to HDAC remains unknown.

The NE is a contiguous structure of the ER. The NE contains many integral proteins (23–26) that participate in genome management or NE reassembly (25, 27, 28). Emerin is a NE inner membrane protein that interacts with A- and B-type lamins to mediate the anchorage of NE to cytoskeleton (29) and to regulate gene transcription by binding to other chromatin-remodeling molecules such as β-catenin or barrier-to-autointegration factor

Significance

The endoplasmic reticulum (ER), although functioning as protein synthesis machinery in the cell, plays other important roles that have yet to be fully unveiled. We found here that the ER can directly send an envoy protein, the sigma-1 receptor (Sig-1R), to the nuclear envelope (NE), where the Sig-1R begins to recruit chromatin-remodeling molecules through the NE integral protein emerin to control gene transcription. Thus, the Sig-1R represents a molecule that shapes the functional connection between the NE and the DNA. We also demonstrate in this study that cocaine, a Sig-1R agonist, down-regulates the critical enzyme monoamine oxidase B that influences the cocaine-induced dopamine level in a dopamine transporter-independent manner via this never-before-described, to our knowledge, Sig-1R-linked genomic action of cocaine.

Author contributions: S.-Y.A.T., J.-Y.C., Z.-X.X., W.-C.C., A.B., and T.-P.S. designed research; S.-Y.A.T., J.-Y.C., M.-S.T., and X.-f.W. performed research; J.-J.H. and W.-C.C. contributed new reagents/analytic tools; S.-Y.A.T., J.-Y.C., Z.-X.X., J.-J.H., W.-C.C., A.B., and T.-P.S. analyzed data; and S.-Y.A.T., J.-Y.C., Z.-X.X., A.B., and T.-P.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

[1]S-Y.A.T. and J.-Y.C. contributed equally to this work.

[2]To whom correspondence should be addressed. Email: TSU@intra.nida.nih.gov.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1518894112/-/DCSupplemental.

(BAF) (23, 30). Emerin also interacts with HDAC3 to regulate the HDAC3 localization and activity at the nuclear periphery (24, 31).

We found here that the Sig-1R serves an envoy protein from the ER to the NE. At the NE, the Sig-1R binds emerin and recruits a complex of chromatin-remodeling molecules to regulate the transcription of a gene that is known to be keen to the action of cocaine: the monoamine oxidase B (MAOB). The results are presented here.

Results

Sig-1R Translocates to the NE of a Cell and Interacts with Nuclear Scaffold Protein Lamin A/C. In this report, we first used NG108 cells (16) and Neuro2A (32) as model cellular systems to provide critical information.

The confocal microscopic examinations confirmed that Sig-1Rs exist primarily at the ER, especially at the perinuclear region (in plane 3, Fig. 1*A*), but also at the NE and in part at the nuclear pore complex, with Ran BP2 as the marker (Fig. 1*A*, images). A NE marker, lamin A/C, was used to further confirm the NE localization of Sig-1R by confocal microscopy (Fig. 1*B*, *a*) and by acquiring the 3D image stacks through an imaging system (*SI Appendix*, Fig. S1*B*). Results indicated that Sig-1Rs colocalized with lamin A/C in sections 10–17 (*SI Appendix*, Fig. S1), specifically indicated as such at section 11 in the cross-sectional intensity scanning (Fig. 1*B*, *b*). Cocaine is an Sig-1R agonist. After cells were treated with cocaine for 30 min, increased colocalization of Sig-1R and lamin A/C was seen at the folds of NE (Fig. 1*C*, *a*) and in multiple focal sections of the nucleus (*SI Appendix*, Fig. S1*C*), specifically shown as the increased relative intensity of Sig-1R over lamin A/C in the comparable section 11 of the NE region (Fig. 1*C*, *b* vs. Fig. 1*B*, *b*). The increased presence of Sig-1Rs at the nucleus caused by cocaine was blocked by a Sig-1R antagonist, BD1063 (Fig. 1*D*). A subcellular fractionation suitable for the detection of NE was thus performed (33). The presence of Sig-1Rs in the NE fraction was significantly increased by cocaine (Fig. 1*E*).

Sig-1R Apparently Interacts Specifically with a NE Integral Protein Emerin. The procedures for producing the NE given in the previous section are not practical (see *SI Appendix*, *Materials and Methods* for reasons) as the starting material for the immunoprecipitation (IP) experiments, which were performed extensively in the rest of this report (except the Sig-1R and BiP experiment in this section). Instead, we pre-cross-linked cellular components with dithiobis (succinimidyl propionate), prepared the nuclei pellet (free of NE; *SI Appendix*, Fig. S2) (34, 35), and solubilized proteins from pellet as the starting material for all IP experiments in this report.

In alignment with our previous finding, cocaine was found to dose-dependently increase the dissociation of the Sig-1R from its cognate binding partner, BiP (Fig. 2*A*). The fluorescence microscopic images also showed that cocaine decreased the colocalization between Sig-1R and BiP (*SI Appendix*, Fig. S3). Interestingly, cocaine concomitantly increased the co-IP between Sig-1Rs and lamin A/C (Fig. 2*B*). Those results prompted us to see whether Sig-1Rs might interact with the NE integral protein emerin.

At the NE, emerin is known to regulate gene expression by affecting the chromatin compaction (36). By using the in vitro His-tag pull-down assay (Fig. 2*C*, *a*), as well as the Sig-1R-EYFP assay (Fig. 2*C*, *b*), we found that Sig-1R associated with emerin in Neuro2A cells. Because cocaine increases the NE localization of Sig-1R, we examined whether cocaine affects the interaction between the Sig-1R and emerin. Indeed, cocaine caused an enhanced interaction between Sig-1Rs and emerin (Fig. 2 *C*, *a* and *b*). Confocal images also indicated an intensified overlay of Sig-1Rs and emerin at perinuclear areas in cocaine-treated cells compared with in controls (Fig. 2*D*). To further confirm that Sig-1Rs form a complex with emerin and lamin A/C, a 2D blue native polyacrylamide gel electrophoresis (BN-PAGE)/SDS/PAGE was performed in which multiprotein complexes are separated under native conditions by BN-PAGE in the first dimension (Fig. 2*E*),

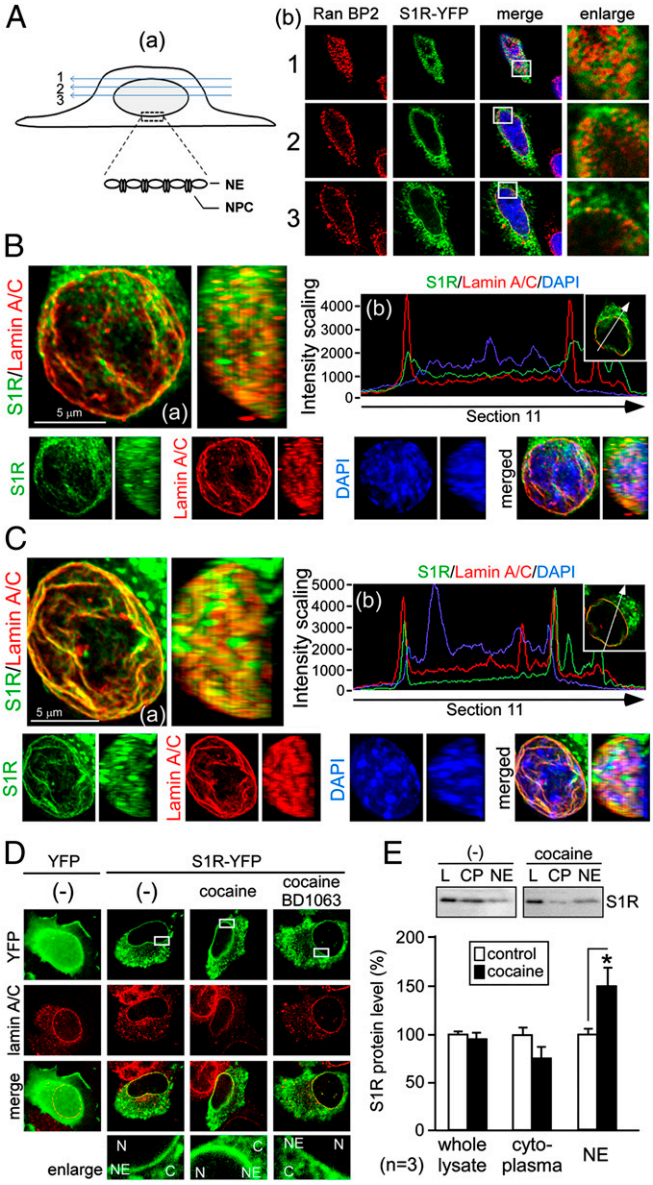

Fig. 1. Sig-1R targets the NE in cocaine-treated cells. (*A*, *a*) Diagram illustrates the confocal laser scanning of individual planes (1–3) of the nucleus. (*A*, *b*) Colocalization of endogenous Sig-1R with nuclear pore complex (on NE) marker RanBP2 in NG108 cells. Sig-1R, green; RanBP2, red; DNA, blue. (*B* and *C*) The whole-nucleus image analysis of Sig-1R-YFP and lamin A/C in differentiated Neuro2A cells under basal condition (*B*) or cocaine (5 μM) treatment for 1 h (*C*). In *B*, *a* and *C*, *a*, left panels are the top-down view (*z*-axis), and right panels are a side view (*x*-axis) of the 3D reconstruction of images. (*B*, *b* and *C*, *b*) Intensity scaling of a comparable section (section 11) taken from *SI Appendix*, Fig. S1 *B* and *C*, respectively. Note the increase of relative intensity at the NE in cocaine-treated cells. (*D*) Sig-1R localization on NE in NG108 cells is intensified by cocaine: the effect of cocaine (5 μM, 1 h) is blocked by the Sig-1R antagonist BD1063 (100 nM). (*E*) Fractionation study on the existence of Sig-1Rs at the nuclear membrane. Neuro2A cells were treated with saline (–) or cocaine for 1 h. Cellular homogenates were prepared for the fractionation assay as described in *SI Appendix*, *Materials and Methods*. $n = 3$ independent experiments (*t* test; $*P < 0.05$).

whereas individual proteins of a complex are separated under denatured conditions and seen through immunoblotting in the second dimension via SDS/PAGE (Fig. 2*F*). The results indicated that emerin, Sig-1R, and lamin A/C were detectable in one vertical line (Fig. 2*F*). Cocaine apparently increased the levels of Sig-1R, lamin A/C, and emerin (Fig. 2*H*). Those

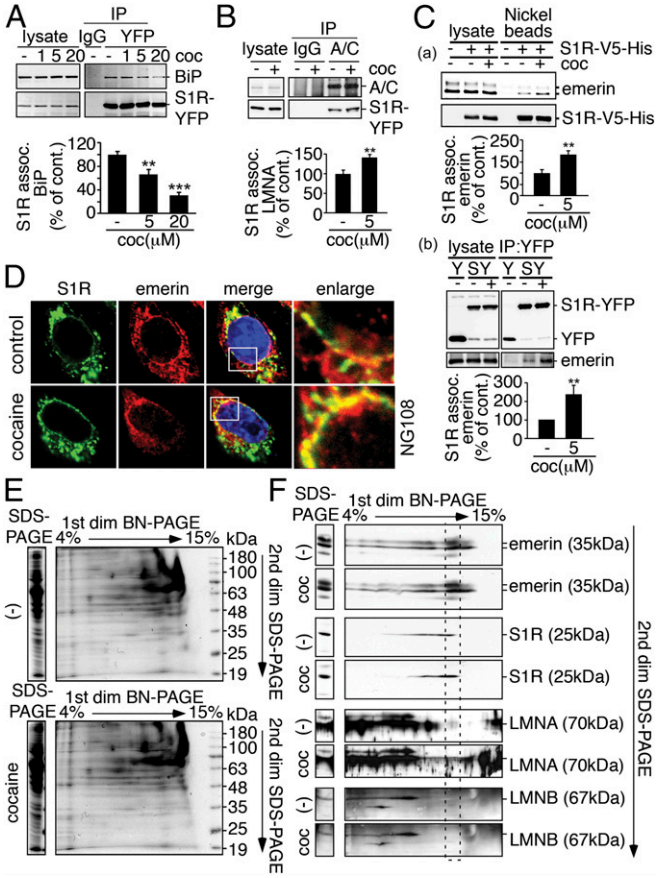

Fig. 2. Sig-1R interacts with the NE protein emerin, and cocaine intensifies the interaction. (*A*) Cocaine causes the dissociation of Sig-1R from BiP in a dose-dependent manner. Sig-1R-YFP-expressing Neuro2A cells were treated with saline or different doses of cocaine (0, 1, 5, 20 μM) for 1 h. Cell lysates were immunoprecipitated with anti-GFP/YFP antibodies followed by immunoblot. Bars represent means ± SEM from three independent experiments (*t* test: $^{**}P < 0.01$; $^{***}P < 0.001$). (*B*) Sig-1R association with lamin A/C is intensified by cocaine. The IP was with anti-lamin A/C antibodies. Bars represent means ± SEM from three independent experiments (*t* test: $^{**}P < 0.01$). (*C*) Cocaine intensifies the Sig-1R-emerin interaction. Sig-1R-V5-His-expressing (*a*) and YFP- or Sig-1R-YFP-expressing (*b*) Neuro2A cells were treated with saline or cocaine (5 μM) for 1 h before extraction and assayed respectively in the IP and blotting. Bars represent means ± SEM from three independent experiments (*t* test: $^{**}P < 0.01$). (*D*) Confocal images of the colocalization of Sig-1R and emerin in NG-108 cells in the absence or presence of cocaine (5 μM, 1 h). (*E* and *F*) Protein complex determination by the native blue gel method. (*E*) Neuro2A cells were treated with saline or cocaine (5 μM) for 1 h. Cell lysates were subjected to 1D SDS/PAGE (*Left*) or 2D BN-PAGE/SDS/PAGE (*Right*). Coomassie blue staining is shown. (*F*) Immunoblot assay after the 1D SDS/PAGE (*Left*) or the 2D BN-PAGE/SDS/PAGE (*Right*) by using respective antibodies to identify and analyze for components of the protein complex. Cocaine did not change the pattern of emerin, but apparently increased the complex formation among emerin, Sig-1R, and lamin A/C.

results demonstrate that cocaine increases the association among Sig-1R, emerin, and lamin A/C.

Although Sig-1Rs co-IPed with emerin and lamin A/C, Sig-1Rs did not co-IP nor colocalize with another NE protein, lamin B receptor (LBR) (*SI Appendix*, Fig. S4 *A* and *C*), and did not co-IP with the transcription factor methyl-CpG-binding protein-2 (MeCP2) either (*SI Appendix*, Fig. S4*A*). The co-IP of Sig-1R and emerin was also confirmed in the nucleus accumbens and striatum of rat brain (*SI Appendix*, Fig. S4*B*).

Emerin Recruits HDAC1 and HDAC2: Cocaine Increases Their Interaction. Emerin was recently shown to influence epigenetic regulation by binding to HDAC3 (24). To clarify whether emerin also binds with other class I HDACs, we performed co-IP assays for emerin, HDAC1, and HDAC2. Anti-emerin antibody pulled down both HDAC1 and HDAC2 (*SI Appendix*, Fig. S5*A*). Conversely, the HDAC2 antibody pulled down emerin and HDAC1 (*SI Appendix*, Fig. S5*B*). Those results indicated that emerin interacts with HDAC1 and HDAC2, as well as with the recently reported HDAC3. Cocaine dose-dependently increased the interaction of emerin with both HDAC1 (*SI Appendix*, Fig. S5*C*) and HDAC2 (*SI Appendix*, Fig. S5*D*).

The Sig-1R/Emerin/BAF/HDACs Complex: Cocaine Enhances the Complex Formation in a Sig-1R-Dependent Manner. Because Sig-1R interacts with emerin, and because emerin was reported to bind HDAC3 (31), we examined whether Sig-1R may interact with HDACs. Indeed, the Sig-1R interacted with HDAC1, HDAC2, and HDAC3 (Fig. 3*A*).

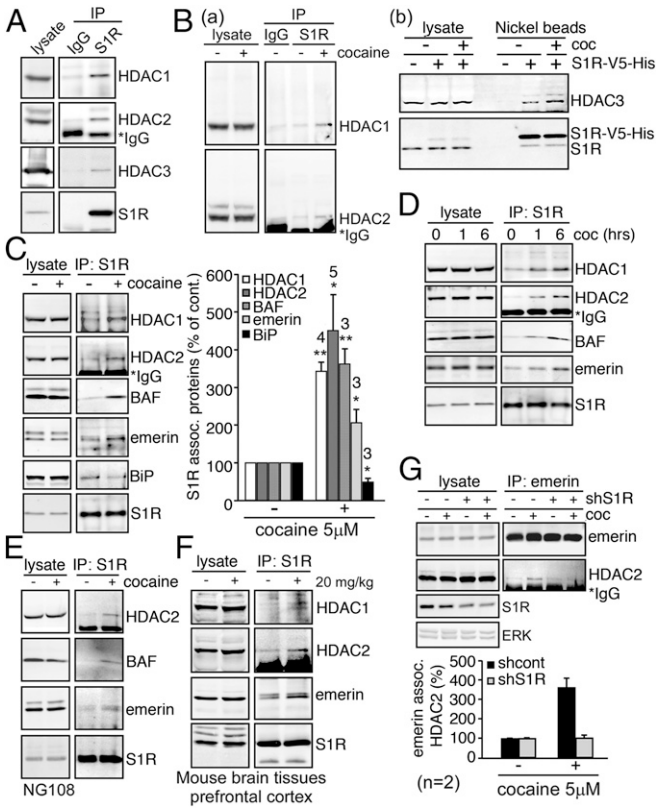

Fig. 3. Sig-1R recruits the emerin/BAF/HDAC complex: cocaine intensifies the complex formation in a Sig-1R-dependent manner. (*A*–*D*) Sig-1R interaction with HDAC1, HDAC2, HDAC3: cocaine increases the interaction. (*A*) Endogenous Sig-1R interaction with HDAC1 and HDAC2 in Neuro2A cells. (*B*) Cocaine increases the interaction between Sig-1R and HDACs. Cocaine's (5 μM, 1 h) effects on the association between endogenous Sig-1R and HDAC1 and HDAC2 (*a*) and between Sig-1R-V5-His and HDAC3 (*b*). (*C* and *D*) Sig-1R forms a complex with HDACs, BAF, and emerin: cocaine (5 μM, 1 h) increases the complex formation. (*C*) Endogenous Sig-1R binds HDAC1, HDAC2, BAF, and emerin; Bip is also shown. Bar represents means ± SEM from three to five independent experiments. Cocaine, as expected, decreases the interaction between Sig-1R and BiP (*t* test: $^{*}P < 0.05$; $^{**}P < 0.01$). (*D*) Time-dependent formation of the complex induced by cocaine (5 μM). (*E* and *F*) Cocaine enhances the interaction between endogenous Sig-1R and indicated proteins in NG108 cells (*E*) and in the prefrontal cortex of mouse brain (16 h after a 20 mg/kg cocaine injection, i.p.) (*F*). (*G*) Cocaine-increased interaction between emerin and HDAC2 is blocked by the knockdown of Sig-1Rs. Cells were transfected with adeno-associated virus (AAV)-shSig-1R or control AAV vector for 48 h before treating with saline or cocaine (5 μM) for 1 h. Bar represents means ± range from two independent experiments.

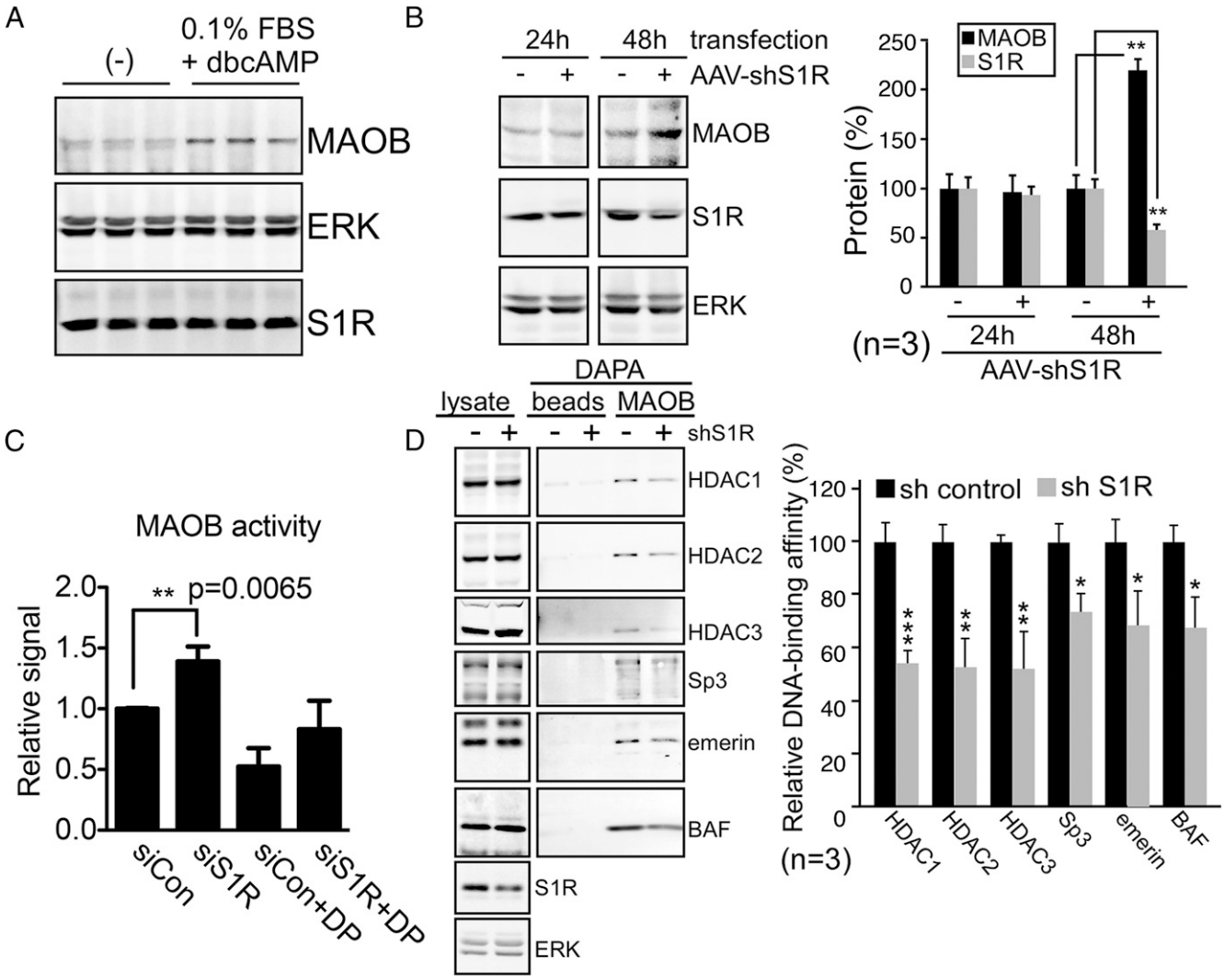

Fig. 4. Sig-1R knockdown increases MAOB protein expression while reducing the binding of the Sig-1R/emerin/BAF/HDAC/specific protein 3 (Sp3) complex to the promoter of MAOB. (*A*) Presence of MAOB in differentiated Neuro2A cells after 2 d of the induction of differentiation. ERK, loading control. (*B*) Increase of MAOB levels in Sig-1R knockdown cells 24 or 48 h after transfection with the AAV-shSig-1R or control AAV vector. (*Right*) Results from three independent experiments (bars = means ± SEM normalized to the ERK loading control; *t* test: $^{**}P < 0.01$). (*C*) Enhanced enzymatic activity of MAOB in Sig-1R knockdown primary neurons. Primary cultured E14 mouse cortical neurons expressing Sig-1R siRNA (siSig-1R) exhibited higher MAOB enzyme activity compared with neurons expressing control siRNA (siCon; *t* test: $^{**}P < 0.01$; means ± SEM; n = 3). R-(−)-deprenyl hydrochloride (DP) reduced MAOB activity in both siCon and siSig-1R neurons. (*D*) Reduction of the complex protein binding to the promoter of MAOB, as seen in the DAPA. Neuro2A cells were transfected with AAV-shSig-1R or control AAV vector for 48 h before assay. The lysates and the products of DAPA were analyzed with immunoblotting by using antibodies as indicated. Beads without DNA probe serves as the negative control. (*Right*) Results from the DNA binding activity of those factors from immunoblotting were quantified and presented as the means ± SEM from three independent experiments (*t* test: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

Further, the interaction between Sig-1Rs and HDAC1, HDAC2, and HDAC3 was enhanced in the presence of cocaine (Fig. 3*B*, *a* and *b*).

BAF was reported to bind emerin and serve as a partner of emerin in helping tether chromatin to the NE (29) as an epigenetic regulator (29, 37). We examined whether the Sig-1R/emerin/HDAC complex may also include BAF. Indeed, as seen from the co-IP assay using Sig-1R antibody, the Sig-1R binds BAF as well as emerin, HDAC1, HDAC2, and Sig-1R's cognate cochaperone BiP (Fig. 3*C*). Cocaine intensified the formation of the Sig-1R/emerin/BAF/HDAC complex while expectedly causing the dissociation of the Sig-1R from BiP (Fig. 3*C*). Cocaine time-dependently increased the complex formation (Fig. 3*D*). The effect of cocaine on the complex formation could be seen in NG108 cells (Fig. 3*E*), as well as in the brain of a mouse that had received 20 mg/kg cocaine (Fig. 3*F*). Importantly, in Sig-1R knockdown cells, cocaine could no longer increase the interaction between emerin and HDAC2 (Fig. 3*G*). Those data suggest cocaine causes the dissociation of Sig-1R from its cognate partner BiP at the ER and begins to cause the translocation of Sig-1Rs from the ER to the NE, where Sig-1Rs recruit chromatin-remodeling proteins for the formation of the Sig-1R/emerin/BAF/HDAC complex.

The Sig-1R/Emerin/BAF/HDAC Complex: Formulation of a Hypothesis on Why Cocaine Initiates the Formation of This Complex. Inasmuch as cocaine caused the translocation of Sig-1Rs from ER to NE to recruit the Sig-1R/emerin/BAF/HDAC complex, we wondered what cocaine might gain in this regard in achieving its addictive property, if any. We previously reported that knockdown of Sig-1Rs in primary neuronal cultures caused an almost 500% increase in the mRNA of MAOB (38), suggesting Sig-1Rs might suppress the gene transcription of MAOB. Now that we have demonstrated that cocaine uses Sig-1Rs to recruit the chromatin-remodeling

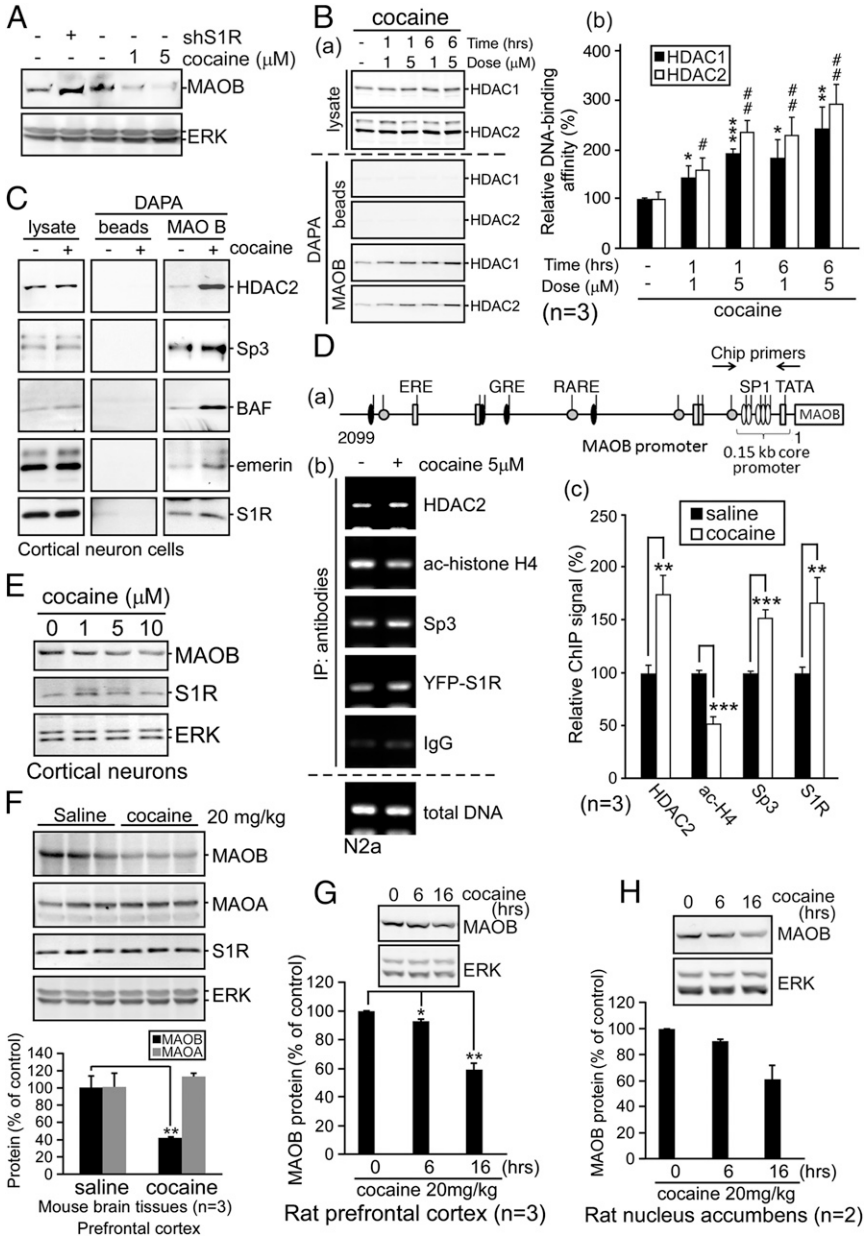

Fig. 5. Cocaine effects on both the MAOB expression and the binding of the Sig-1R-recruited protein complex to the promoter of MAOB. (*A*) Cocaine dose-dependently decreased MAOB protein levels in Neuro2A cells. (*B*, *a*) Cocaine dose-dependently and time-dependently increased HDAC1 and HDAC2 binding to the MAOB promoter: DAPA assays. (*B*, *b*) Quantified results from *B*, *a*. Bar = means ± SEM ($n = 3$ independent experiments (HDAC1 *t* test: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; HDAC2 *t* test: $^{\#}P < 0.05$; $^{\#}P < 0.01$). (*C*) Cocaine (5 μM, 1h) increased the binding of Sig-1R-recruited proteins to the MAOB promoter in mouse primary cortical neurons. (*D*, *a*) Scheme of the chromatin IP analysis. (*D*, *b*) DNA analysis from chromatin IP of the in vivo effect of cocaine on the binding of Sig-1R-recruited proteins to the MAOB promoter in Neuro2A cells. (*D*, *c*) Quantified results from *D*, *b*. Bar = means ± SEM of signals of the PCR products from chromatin IP normalized to the total DNA (*t* test: $^{**}P < 0.01$; $^{***}P < 0.001$; $n = 3$). (*E*–*H*) Ex vivo (*E*) and in vivo (*F*–*H*) examinations of cocaine effects on the MAOB protein levels. (*E*) Primary mouse cortical neurons treated with different doses of cocaine for 18 h before assay. (*F*) Mouse received 20 mg/kg cocaine, and 16 h later the prefrontal cortex of mouse was processed for Western blotting. Bar represents means ± SEM (*t* test: $^{**}P < 0.01$; $n = 3$ independent experiments). (*G* and *H*) Tissues from prefrontal cortex (*G*) and nucleus accumbens (*H*) of rats were obtained at different points after administration of cocaine (20 mg/kg). Bar represents means ± SEM or range from three (*G*, *t* test: $^{*}P < 0.05$; $^{**}P < 0.01$) or two (*H*) independent experiments, respectively.

complex, we hypothesized that cocaine may use this complex to suppress the gene transcription of MAOB. The transcription factor Sp3 may thus be involved for two reasons: Sp3 binds GC-boxes on the MAOB core promoter for the regulation of the MAOB expression (39), and Sp3 recruits HDAC1, HDAC2, or HDAC3 to the promoter for histone deacetylation and gene silencing (40, 41). The following sections provide experimental evidence to support this hypothesis.

Sp3 Links the Sig-1R/Emerin/BAF/HDAC Complex to the MAOB Promoter. We first examined for the protein level of MAOB as it relates to Sig-1Rs. To this end, we used the method that causes the differentiation of Neuro2A cells into dopaminergic cells expressing MAOB (32) (Fig. 4*A*). We found that the protein level of MAOB in the differentiated cells is largely increased in Sig-1R-knockdown cells (Fig. 4*B*). The associated enzymatic activity of MAOB was also increased in Sig-1R knockdown cells (Fig. 4*C*).

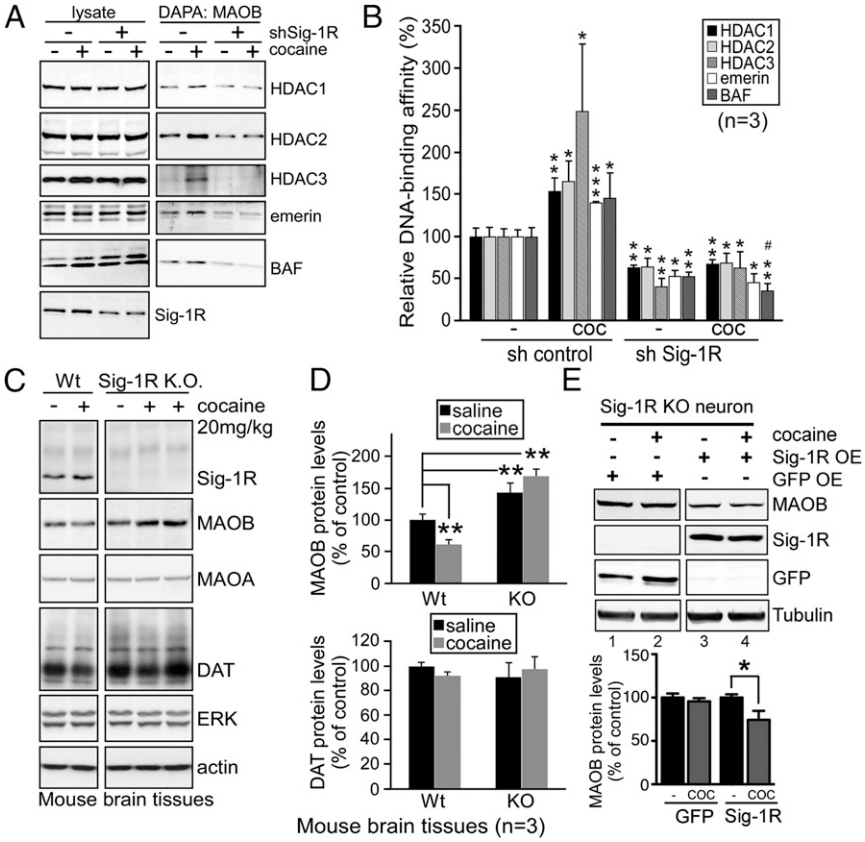

Fig. 6. Sig-1R-dependency of cocaine effects on the formation of the recruited complex and the expression of the MAOB protein. (*A*) Reduced binding of recruited proteins to the MAOB promoter (DAPA assay) in Sig-1R-knockdown NG108 cells and the inability of cocaine (5 μM, 1 h) to enhance the promoter binding of those proteins in those cells. (*B*) Bar represents means ± SEM from three independent experiments from *A* (*t* test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). COC, cocaine. (*C* and *D*) Effects of cocaine (20 mg/kg; 16 h) on the MAOB, MAOA, and DAT levels in wild-type or Sig-1R knockout mice. Prefrontal cortices were examined. Bar represents means ± SEM in *D* (*t* test: $**P < 0.01$; $n = 3$ independent experiments from *C*). (*E*) Exogenous expression of Sig-1Rs in primary neurons from Sig-1R knockout mouse restores cocaine's (1 μM, 24 h) ability to suppress MAOB expression. Neurons were transduced with either AAV-GFP controls (lanes 1 and 2) or AAV-Sig-1R (lanes 3 and 4). Bar represents means ± SEM (*t* test: $*P < 0.05$; $n = 3$ independent experiments).

To establish the potential relation between the Sig-1R/emerin/BAF/HDAC complex and Sp3, we used the biotin-labeled MAOB DNA probes (the Sp binding site of five GC-boxes on the MAOB promoter) in a DNA affinity precipitation assay (DAPA) to examine whether the complex, as well as Sp3, exists on the MAOB promoter. Indeed, HDAC1/2/3, Sp3, emerin, and BAF all existed at the MAOB promoter (Fig. 4*D*). Importantly, knockdown of Sig-1Rs caused a reduction of all of their presence, including that of Sp3, at the MAOB promoter (Fig. 4*D*). Those results, when taken together, suggest that the Sig-1R/emerin/BAF/HDAC complex may in fact bind Sp3, and that Sp3, being targeted by this complex, serves to link the chromatin-remodeling complex to the MAOB promoter. It is thus possible that, through this chromatin-remodeling complex and Sp3, Sig-1R is able to suppress the transcription of MAOB.

Cocaine Increases the Association of Sig-1R/Emerin/BAF/HDAC as Well as Sp3 with the MAOB Promoter. To further confirm the effect of cocaine on MAOB, we first established the fact that cocaine dose-dependently caused a reduction of MAOB proteins in differentiated Neuro2A cells (Fig. 5*A*). Then, the effect of cocaine on the HDAC1 or HDAC2 association with the Sp binding site of the MAOB promoter was examined. As seen in the DAPA assay, which used the Sp binding probe, HDAC1 and HDAC2 binding to the MAOB promoter were time-dependently as well as dose-dependently increased by cocaine in Neuro2A cells (Fig. 5*B*). Further, cocaine also increased the binding of Sp3, BAF, emerin, and Sig-1R to the Sp binding sites of the MAOB promoter (Fig. 5*C*). However, cocaine did not affect the Sp3 level on the Sp-binding sites of the superoxide dismutase promoter, which served as a negative control (*SI Appendix*, Fig. S6). To substantiate those results even further, we performed the in vivo DNA–protein IP assay (CHIP) and found that cocaine increased the existence of Sig-1R, HDAC2, and Sp3 at the MAOB promoter (Fig. 5*D*). However, the existence of acetylated histone-4 at the MAOB promoter was reduced by the treatment of cocaine (Fig. 5*D*), suggesting an enhanced HDAC activity.

The effect of cocaine in reducing the protein expression of MAOB was also seen in primary cortical neurons of mouse (Fig. 5*E*), in the prefrontal cortex of mouse receiving 20 mg/kg of cocaine (Fig. 5*F*; cocaine has no effect on MAOA), and in the rat prefrontal cortex (Fig. 5*G*) and nuclear accumbens (Fig. 5*H*) in a time-dependent manner after the cocaine treatment.

Thus, cocaine attenuates the transcription of MAOB via Sig-1Rs by increasing the binding of Sig-1R/emerin/BAF/HDAC and Sp3 to the promoter of MAOB.

Cocaine-Induced Transcriptional Regulation of MAOB Is Sig-1R-Dependent. In the wild-type NG108 cells, cocaine caused an increase in the binding of HDAC1, HDAC2, HDAC3, emerin, and BAF to the promoter of MAOB, as seen from the DAPA assay (Fig. 6 *A* and *B*). However, in Sig-1R knockdown cells, this effect of cocaine was absent (Fig. 6 *A* and *B*). In wild-type mouse, cocaine caused a decrease of MAOB in the prefrontal cortex

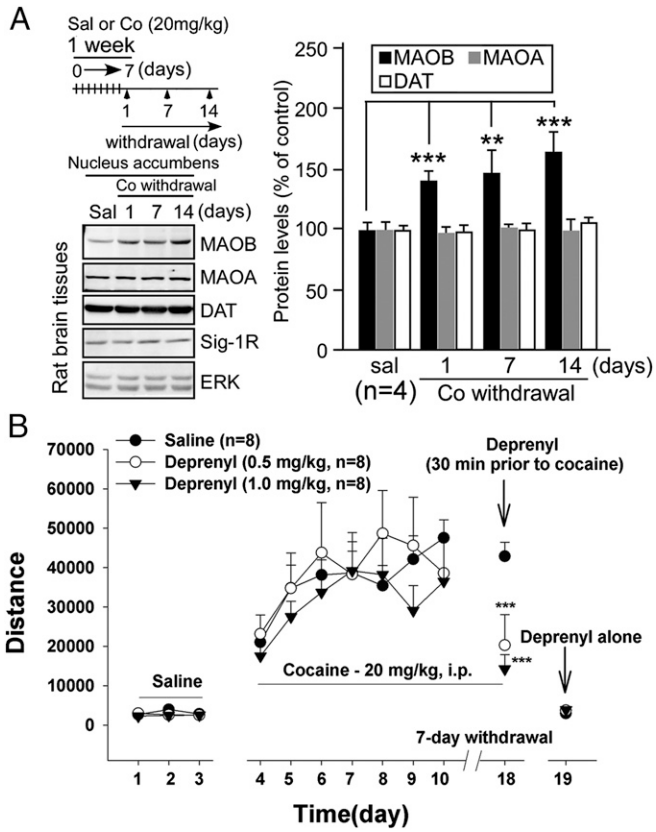

Fig. 7. Cocaine withdrawal increases MAOB at the nucleus accumbens: the MAOB inhibitor deprenyl blocks cocaine's action in behaviorally sensitized rats. Rats received daily injections of cocaine (20 mg/kg of cocaine, i.p.) for 7 d and were examined for locomotion daily. Rats then were withdrawn from cocaine for 1, 7, and 14 d. On day 7 after withdrawal, rats received deprenyl (0.5 mg/kg or 1.0 mg/kg, i.p.) 30 min before test for cocaine-induced locomotion. (*A*) MAOB, MAOA, and DAT protein levels at the nucleus accumbens during withdrawal from cocaine. Bars represent means ± SEM ($n = 4$ independent experiments from four rats; *t* test, $^{**}P < 0.01$; $^{***}P < 0.001$). (*B*) Repeated cocaine injections produced significant locomotor sensitization in all three groups of rats before deprenyl pretreatment. On the test day (day 18; i.e., 7 d after withdrawal), pretreatment with deprenyl (0.5, 1.0 mg/kg, i.p.) dose-dependently blocked cocaine-induced increase in locomotion and locomotor sensitization ($F_{2,21} = 6.74$, $P < 0.01$, one-way ANOVA; $^{***}P < 0.001$, *t* test). Deprenyl alone (0.5, 1.0 mg/kg, i.p.), given a day after the cocaine blockade test, did not affect the locomotion.

(Fig. 6 *C* and *D*). In Sig-1R knockout mouse, the MAOB level was increased when compared with that seen in wild-type mouse (Fig. 6 *C* and *D*). Cocaine was, however, unable to decrease the MAOB level in the brain of the Sig-1R knockout mouse (Fig. 6 *C* and *D*). Although cocaine tended to increase the MAOB level in Sig-1R knockout mouse, the difference did not reach statistical significance (Fig. 6*D*). Cocaine did not affect the gene expression of MAOA or a dopamine (DA) transporter in the present studies (Fig. 6 *C* and *D*). In addition, to provide a causal role of Sig-1R in cocaine-suppressed MAOB level, we overexpressed Sig-1Rs in Sig-1R knockout neurons and found that Sig-1R overexpression restored the cocaine's ability to suppress the MAOB level in those neurons (Fig. 6*E*).

Withdrawal from Chronic Cocaine Increases MAOB; the MAOB Inhibitor Deprenyl Blocks Cocaine Effect in Behaviorally Sensitized Rats During Withdrawal. We performed the chronic cocaine study in rats to validate the physiological relevance of the present in vitro and in vivo data, specifically regarding the level of MAOB affected by cocaine. Rats received a daily injection of cocaine (20 mg/kg, i.p.) for 7 d and were withdrawn from cocaine for 1, 7, and 14 d. Rats were tested for behavioral sensitization to cocaine on each day of the cocaine injection, as well as on day 7 after cocaine withdrawal. The MAOB level at nucleus accumbens was examined by Western blotting.

We found that the MAOB level was increased throughout the days (day 1, 7, 14) of examination during withdrawal from cocaine (Fig. 7*A*). This result suggested a transcriptional rebound of MAOB in the absence of cocaine. Thus, we tested whether the MOAB inhibitor deprenyl may block the behavioral sensitization to cocaine on day 7 after withdrawal. Indeed, although deprenyl by itself had no effect in the absence of cocaine, deprenyl (at 0.5 mg/kg or 1 mg/kg, i.p.) blocked the behavioral sensitization action of cocaine (Fig. 7*B*).

Discussion

Sig-1Rs have been implicated in many physiological functions (3, 10, 13, 42–47). Our results from the present study add an additional function of Sig-1R, in that it regulates gene expression at the NE.

The Sig-1R represents a newly recognized NE protein in the living system (17). Although we used cocaine as a Sig-1R agonist to increase the Sig-1R at the NE, we noted that, in alignment with a recent report (17), some Sig-1Rs exist at the NE even in the absence of cocaine (Fig. 1 *A* and *C*). This result suggests the possibility that endogenous Sig-1R ligands such as dehydroepiandrosterone sulfate (5), N,*N*-dimethyltryptamine (7), or myristic acid (47) may play a role in translocating Sig-1Rs into the NE. This regulation of Sig-1R on gene transcription is physiologically relevant because in Sig-1R-knockdown cells or knockout mice, an increased level of the MAOB protein was seen. Thus, the MAOB gene is perhaps normally suppressed via its linkage to the Sig-1R-recruited chromatin-remodeling complex. A proposed mechanism is shown (*SI Appendix*, Fig. S7).

Cocaine's action in the brain involves its effect on the DA concentration (48) in the synaptic cleft, which is generally attributed to cocaine blocking the DA uptake at the DA transporter (DAT) (49–51). Our data in this study suggest that, in addition to the DAT, cocaine may regulate the DA concentration by suppressing the gene expression of the DA-degrading enzyme MAOB via this genomic means by translocating Sig-1Rs from the ER into the NE to suppress the transcription of MAOB. Thus, our finding sheds new light on the mode of action of cocaine. However, inasmuch as the concentration of cocaine used in this study is in the micromole range, even though we have clearly showed that cocaine lost its effect in Sig-1R knockdown or knockout systems, the possibility that DAT may involve in those events cannot be totally ruled out at present.

Our result of the effect of the MAOB inhibitor deprenyl on blocking cocaine-induced behavioral sensitization during cocaine withdrawal is striking (Fig. 7*B*). However, an apparent paradox exists in theory: It is understandable that decreasing MAOB after cocaine treatment should enhance the acute action of cocaine, and yet, on the contrary, deprenyl blocked the action of cocaine during withdrawal. We can only speculate at present that deprenyl, given before cocaine during withdrawal, should have raised up the localized basal level of DA that is known to compete with cocaine at DAT as a competitive inhibitor (52, 53), thus attenuating the action of cocaine. Further study is certainly warranted in this regard.

Although deprenyl was not apparently effective in attenuating ongoing cocaine use in a double-blind, placebo clinical trial (54), to the best of our knowledge, deprenyl has never been tested for reducing patient's cocaine use after a period of withdrawal from cocaine. In fact, such a use of deprenyl during cocaine withdrawal was suggested at the end of the same report

(54). Our results in the present study with deprenyl support such a notion.

Materials and Methods

Immunofluorescence and Confocal Microscope. Cells were fixed with 4% (wt/vol) paraformaldehyde (Sigma-Aldrich) in PBS according to a method described previously (3). Details are described in the *SI Appendix, Materials and Methods*.

Fractionation for the NE. The subcellular fractionation was performed according to the Matunis' protocol, with a slight modification (33).

BN-PAGE. BN-PAGE assay was performed according to a method described by Fiala et al. (55) and Wittig et al. (56). Details are described in *SI Appendix, Materials and Methods*.

Chromatin IP Assay. Chromatin IP assay was performed according to a method described previously (57). Cells were fixed with 1% (wt/vol) formaldehyde and then harvested and sonicated to generate chromatin fragments between 200 and 500 bp. The fixed DNA–protein complex were immunoprecipitated with normal rabbit IgG, anti-Sp3, anti-HDAC2, anti-GFP/YFP, or anti-acetyl-Histone H4 (Millipore) antibodies. Immunoprecipitated DNA was analyzed by PCR. The primer sequences for promoter of mouse MAOB in PCR analyses were as follows: 5′-AGGGTCTTGACCCCTGGG and 5′-TAAGCTGGAGGGCCGCCT.

Statistical Analyses. The statistical analyses for the MAOB enzymatic activity, as well as for all data of the Western blotting, DAPA assays, and CHIP assays, were performed by the unpaired two-tailed Student *t* test with significant level set at $P < 0.05$. The main effect in the behavioral sensitization test was analyzed by one-way ANOVA, whereas the *P* value for the group comparison was analyzed by the Student-Newman-Keuls *t* test.

ACKNOWLEDGMENTS. We thank Brandon Harvey of the National Institute on Drug Abuse, Intramural Research Program for the supply of adeno-associated virus (AAV)-GFP and adeno-associated virus (AAV)-Sig-1R, and Diana Martinez, Roy Wise, and Yavin Shaham for helpful discussions. This work was supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, US Department of Health and Human Services (S.-Y.A.T., J.-Y.C., M.-S.T., X.-f.W., Z.-X.X., A.B., and T.-P.S.). Supported was also provided by Grants MOST 103-2320-B-038-046 and MOST 103-2321-B-038-001 from the Ministry of Science and Technology, Taiwan (to J.-Y.C., J.-J.H., and W.-C.C.).

1. Stathopulos PB, et al. (2013) STIM1/Orai1 coiled-coil interplay in the regulation of store-operated calcium entry. *Nat Commun* 4:2963.
2. de Brito OM, Scorrano L (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456(7222):605–610.
3. Hayashi T, Su TP (2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell* 131(3):596–610.
4. Boehning D, et al. (2003) Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. *Nat Cell Biol* 5(12):1051–1061.
5. Su TP, London ED, Jaffe JH (1988) Steroid binding at sigma receptors suggests a link between endocrine, nervous, and immune systems. *Science* 240(4849):219–221.
6. Snyder SH, Largent BL (1989) Receptor mechanisms in antipsychotic drug action: Focus on sigma receptors. *J Neuropsychiatry Clin Neurosci* 1(1):7–15.
7. Fontanilla D, et al. (2009) The hallucinogen N,N-dimethyltryptamine (DMT) is an endogenous sigma-1 receptor regulator. *Science* 323(5916):934–937.
8. Ortega-Roldan JL, Ossa F, Schnell JR (2013) Characterization of the human sigma-1 receptor chaperone domain structure and binding immunoglobulin protein (BiP) interactions. *J Biol Chem* 288(29):21448–21457.
9. Hedskog L, et al. (2013) Modulation of the endoplasmic reticulum-mitochondria interface in Alzheimer's disease and related models. *Proc Natl Acad Sci USA* 110(19):7916–7921.
10. Mori T, Hayashi T, Hayashi E, Su TP (2013) Sigma-1 receptor chaperone at the ER-mitochondrion interface mediates the mitochondrion-ER-nucleus signaling for cellular survival. *PLoS One* 8(10):e76941.
11. Su TP, Hayashi T, Maurice T, Buch S, Ruoho AE (2010) The sigma-1 receptor chaperone as an inter-organelle signaling modulator. *Trends Pharmacol Sci* 31(12):557–566.
12. Mavlyutov TA, et al. (2013) Lack of sigma-1 receptor exacerbates ALS progression in mice. *Neuroscience* 240:129–134.
13. Balasuriya D, Stewart AP, Edwardson JM (2013) The σ-1 receptor interacts directly with GluN1 but not GluN2A in the GluN1/GluN2A NMDA receptor. *J Neurosci* 33(46): 18219–18224.
14. Kim FJ, et al. (2010) Sigma 1 receptor modulation of G-protein-coupled receptor signaling: Potentiation of opioid transduction independent from receptor binding. *Mol Pharmacol* 77(4):695–703.
15. Kourrich S, et al. (2013) Dynamic interaction between sigma-1 receptor and Kv1.2 shapes neuronal and behavioral responses to cocaine. *Cell* 152(1-2):236–247.
16. Hayashi T, Su TP (2003) Intracellular dynamics of sigma-1 receptors (sigma(1) binding sites) in NG108-15 cells. *J Pharmacol Exp Ther* 306(2):726–733.
17. Mavlyutov TA, Epstein M, Guo LW (2015) Subcellular localization of the sigma-1 receptor in retinal neurons - an electron microscopy study. *Sci Rep* 5:10689.
18. Kornberg MD, et al. (2010) GAPDH mediates nitrosylation of nuclear proteins. *Nat Cell Biol* 12(11):1094–1100.
19. Rogge GA, Wood MA (2013) The role of histone acetylation in cocaine-induced neural plasticity and behavior. *Neuropsychopharmacology* 38(1):94–110.
20. Malvaez M, et al. (2013) HDAC3-selective inhibitor enhances extinction of cocaine-seeking behavior in a persistent manner. *Proc Natl Acad Sci USA* 110(7):2647–2652.
21. Kennedy PJ, et al. (2013) Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. *Nat Neurosci* 16(4):434–440.
22. Robison AJ, Nestler EJ (2011) Transcriptional and epigenetic mechanisms of addiction. *Nat Rev Neurosci* 12(11):623–637.
23. Gruenbaum Y, Margalit A, Goldman RD, Shumaker DK, Wilson KL (2005) The nuclear lamina comes of age. *Nat Rev Mol Cell Biol* 6(1):21–31.
24. Demmerle J, Koch AJ, Holaska JM (2012) The nuclear envelope protein emerin binds directly to histone deacetylase 3 (HDAC3) and activates HDAC3 activity. *J Biol Chem* 287(26):22080–22088.
25. Akhtar A, Gasser SM (2007) The nuclear envelope and transcriptional control. *Nat Rev Genet* 8(7):507–517.
26. Bermejo R, Kumar A, Foiani M (2012) Preserving the genome by regulating chromatin association with the nuclear envelope. *Trends Cell Biol* 22(9):465–473.
27. Asencio C, et al. (2012) Coordination of kinase and phosphatase activities by Lem4 enables nuclear envelope reassembly during mitosis. *Cell* 150(1):122–135.
28. Turgay Y, et al. (2014) SUN proteins facilitate the removal of membranes from chromatin during nuclear envelope breakdown. *J Cell Biol* 204(7):1099–1109.
29. Berk JM, et al. (July 22, 2014) Emerin intermolecular links to emerin and BAF. *J Cell Sci*, 10.1242/jcs.148247.
30. Ho CY, Jaalouk DE, Vartiainen MK, Lammerding J (2013) Lamin A/C and emerin regulate MKL1-SRF activity by modulating actin dynamics. *Nature* 497(7450):507–511.
31. Demmerle J, Koch AJ, Holaska JM (2013) Emerin and histone deacetylase 3 (HDAC3) cooperatively regulate expression and nuclear positions of MyoD,Myf5, and Pax7 genes during myogenesis. *Chromosome Res* 21(8):765–779.
32. Tremblay RG, et al. (2010) Differentiation of mouse Neuro 2A cells into dopamine neurons. *J Neurosci Methods* 186(1):60–67.
33. Matunis MJ (2006) Isolation and fractionation of rat liver nuclear envelopes and nuclear pore complexes. *Methods* 39(4):277–283.
34. Buch C, et al. (2009) An integral protein of the inner nuclear membrane localizes to the mitotic spindle in mammalian cells. *J Cell Sci* 122(Pt 12):2100–2107.
35. Jafferali MH, et al. (2014) MCLIP, an effective method to detect interactions of transmembrane proteins of the nuclear envelope in live cells. *Biochim Biophys Acta* 1838(10):2399–2403.
36. Schneider R, Grosschedl R (2007) Dynamics and interplay of nuclear architecture, genome organization, and gene expression. *Genes Dev* 21(23):3027–3043.
37. Bar DZ, et al. (2014) BAF-1 mobility is regulated by environmental stresses. *Mol Biol Cell* 25(7):1127–1136.
38. Tsai SY, Rothman RK, Su TP (2012) Insights into the Sigma-1 receptor chaperone's cellular functions: A microarray report. *Synapse* 66(1):42–51.
39. Wong WK, Chen K, Shih JC (2001) Regulation of human monoamine oxidase B gene by Sp1 and Sp3. *Mol Pharmacol* 59(4):852–859.
40. Wilson AJ, et al. (2006) Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer. *J Biol Chem* 281(19):13548–13558.
41. Won J, Yim J, Kim TK (2002) Sp1 and Sp3 recruit histone deacetylase to repress transcription of human telomerase reverse transcriptase (hTERT) promoter in normal human somatic cells. *J Biol Chem* 277(41):38230–38238.
42. Pal A, et al. (2012) The sigma-1 receptor protects against cellular oxidative stress and activates antioxidant response elements. *Eur J Pharmacol* 682(1-3):12–20.
43. Tsai SY, et al. (2009) Sigma-1 receptors regulate hippocampal dendritic spine formation via a free radical-sensitive mechanism involving Rac1xGTP pathway. *Proc Natl Acad Sci USA* 106(52):22468–22473.
44. Kourrich S, Su TP, Fujimoto M, Bonci A (2012) The sigma-1 receptor: Roles in neuronal plasticity and disease. *Trends Neurosci* 35(12):762–771.
45. Mavlyutov TA, Epstein ML, Andersen KA, Ziskind-Conhaim L, Ruoho AE (2010) The sigma-1 receptor is enriched in postsynaptic sites of C-terminals in mouse motoneurons. An anatomical and behavioral study. *Neuroscience* 167(2):247–255.
46. Pabba M, et al. (2014) NMDA receptors are upregulated and trafficked to the plasma membrane after sigma-1 receptor activation in the rat hippocampus. *J Neurosci* 34(34):11325–11338.
47. Tsai SY, Pokrass MJ, Klauer NR, Nohara H, Su TP (2015) Sigma-1 receptor regulates Tau phosphorylation and axon extension by shaping p35 turnover via myristic acid. *Proc Natl Acad Sci USA* 112(21):6742–6747.
48. Ranaldi R, Wise RA (2001) Blockade of D1 dopamine receptors in the ventral tegmental area decreases cocaine reward: Possible role for dendritically released dopamine. *J Neurosci* 21(15):5841–5846.
49. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237(4819): 1219–1223.
50. Volkow ND, et al. (1997) Relationship between subjective effects of cocaine and dopamine transporter occupancy. *Nature* 386(6627):827–830.
51. Hong WC, Amara SG (2010) Membrane cholesterol modulates the outward facing conformation of the dopamine transporter and alters cocaine binding. *J Biol Chem* 285(42):32616–32626.

52. Calligaro DO, Eldefrawi ME (1987-1988) High affinity stereospecific binding of [3H] cocaine in striatum and its relationship to the dopamine transporter. *Membr Biochem* 7(2):87–106.
53. Beuming T, et al. (2008) The binding sites for cocaine and dopamine in the dopamine transporter overlap. *Nat Neurosci* 11(7):780–789.
54. Elkashef A, et al. (2006) Double-blind, placebo-controlled trial of selegiline transdermal system (STS) for the treatment of cocaine dependence. *Drug Alcohol Depend* 85(3):191–197.
55. Fiala GJ, Schamel WW, Blumenthal B (2011) Blue native polyacrylamide gel electrophoresis (BN-PAGE) for analysis of multiprotein complexes from cellular lysates. *J Vis Exp* (48):e2164.
56. Wittig I, Braun HP, Schägger H (2006) Blue native PAGE. *Nat Protoc* 1(1):418–428.
57. Chuang JY, et al. (2012) Sp1 phosphorylation by cyclin-dependent kinase 1/cyclin B1 represses its DNA-binding activity during mitosis in cancer cells. *Oncogene* 31(47): 4946–4959.