Published in final edited form as:

Neurochem Int. 2011 October ; 59(5): 610–617. doi:10.1016/j.neuint.2011.04.013.

Cocaine increases phosphorylation of MeCP2 in the rat striatum *in vivo*: a differential role of NMDA receptors

Li-Min Mao¹, Erica Horton², Ming-Lei Guo¹, Bing Xue¹, Dao-Zhong Jin¹, Eugene E. Fibuch², and John Q. Wang^{1,2,*}

¹ Department of Basic Medical Science, School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA

² Department of Anesthesiology, School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA

Abstract

Methyl CpG-binding protein-2 (MeCP2) is a transcriptional regulator that binds to methylated DNA at CpG sites and functions to silence DNA transcription. MeCP2 is subject to the phosphorylation modification at serine 421 (S421), which releases MeCP2 from DNA and thus facilitates gene expression. As a transcriptional repressor densely expressed in limbic reward circuits of adult mammalian brains, MeCP2 is recently emerging as a critical epigenetic factor in experience-dependent neural plasticity and psychostimulant addiction. In this study, we investigated the regulation of MeCP2 phosphorylation in the rat striatum by the psychostimulant cocaine *in vivo*. We found that acute systemic injection of cocaine increased MeCP2 phosphorylation at S421 in the rat striatum, including both the caudate putamen and the nucleus accumbens, while cocaine did not affect MeCP2 phosphorylation in the medial prefrontal cortex. The cocaine-stimulated MeCP2 phosphorylation in the nucleus accumbens was a rapid and transient event, as it was evident at 20 min and returned to normal levels 3 h after drug injection. The cocaine effect in the caudate putamen was however relatively delayed. Reliable induction of MeCP2 phosphorylation in this region was detected at 60 min. Pretreatment with an *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist significantly reduced the cocaine-stimulated MeCP2 phosphorylation in the caudate putamen, although not in the nucleus accumbens. Our data support that MeCP2 is a sensitive target of psychostimulants. Its phosphorylation status is regulated by psychostimulant exposure. NMDA receptors play a region-specific role in linking cocaine to MeCP2 phosphorylation in striatal neurons *in vivo*.

Keywords

Glutamate; dopamine; stimulant; caudate; nucleus accumbens; prefrontal cortex; transcription; CREB; addiction

© 2011 Elsevier Ltd. All rights reserved.

*Corresponding author: Dr. John Q. Wang, Department of Basic Medical Science, University of Missouri-Kansas City, School of Medicine, 2411 Holmes Street, Kansas City, Missouri 64108, USA, Tel: (816) 235-1786; Fax: (816) 235-5574, wangjq@umkc.edu.

Conflict of interest: there is no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Methyl CpG-binding protein-2 (MeCP2) is a founding member of the DNA-binding protein family that binds to methylated DNA at CpG sites (Lewis et al., 1992; Klose and Bird, 2006). Once bound, MeCP2 recruits a histone deacetylase (HDAC) to silence transcription of target genes. As a global transcription repressor, MeCP2 is important for neural development. Mutations of the gene (*Mecp2*) encoding MeCP2 cause Rett syndrome, an X-linked postnatal neurodevelopmental disorder characterized by autism-like symptoms and mental retardation (Gemelli et al., 2006; Chahrouh and Zoghbi, 2007). Additionally, MeCP2 is densely expressed in postmitotic neurons of adult brains and is considered to be an epigenetic factor critical for the regulation of adult brain function. Indeed, MeCP2 is sensitive to changing neuronal activity and Ca^{2+} signals, and functions to link these changes to altered expression of various genes. For example, the neuronal activity-dependent Ca^{2+} influx induced the *de novo* phosphorylation of MeCP2 at serine 421 (S421) through a CaMKII-dependent mechanism. This phosphorylation contributes to releasing MeCP2 from brain-derived neurotrophic factor (BDNF) promoter III, thereby facilitating BDNF transcription, dendritic growth, and spine maturation (Chen et al., 2003; Martinowich et al., 2003; Zhou et al., 2006). Other studies also support the role of MeCP2 in the regulation of excitatory transmission and learning and memory (Moretti et al., 2006; Nelson et al., 2006; 2008; Chao et al., 2007; Dani and Nelson, 2009; Gambino et al., 2010). Apparently, the MeCP2-mediated stimulus-transcription coupling is an important pathway linking neuronal activity to a program of gene expression. Aberration of this coupling may lead to various neuropsychiatric disorders (Monteggia and Kavalali, 2009).

Psychostimulant drugs of abuse (cocaine and amphetamines) have long been appreciated to induce distinct sets of gene expression in the forebrain reward circuits, including the striatum. Altered gene expression initiates and directs a series of neuroadaptations at cellular and synaptic levels, which contributes to enduring drug-seeking behavior (Chao and Nestler, 2004; McClung and Nestler, 2008). While underlying mechanisms for drug-stimulated gene expression are not fully understood, MeCP2 may play a significant role at the transcriptional level based on several recent studies (Feng and Nestler, 2010). Deng et al. (2010) reported that acute amphetamine increased MeCP2 phosphorylation at S421 in the mouse ventral striatum/nucleus accumbens (NAc). The increased MeCP2 phosphorylation correlated with behavioral sensitization to chronic amphetamine. *Mecp2* hypomorphic mutant mice showed deficit of amphetamine-stimulated structural plasticity and immediate early gene expression in the NAc. Im et al. (2010) found that extended self-administration of cocaine elevated MeCP2 expression in the rat dorsal striatum/caudate putamen (CPu), similar to the early results seen after repeated cocaine injections in rats (Cassel et al., 2006). Knockdown of MeCP2 expression in the CPu through local lentiviral delivery of a short hairpin interfering RNA reversed cocaine intake (Im et al., 2010). These results indicate that MeCP2 is a sensitive target of psychostimulants. Its responses to psychostimulant exposure constitute as an essential element of overall neuroadaptations related to the persistent properties of drugs of abuse.

Since MeCP2 expression and phosphorylation at S421 in response to acute cocaine administration have been less thoroughly investigated and characterized, we carried out this study to define them in rats *in vivo*. We examined the effect of a single and behaviorally active dose of cocaine on total and S421-phosphorylated MeCP2 expression in the three forebrain regions: the CPu, NAc, and medial prefrontal cortex (mPFC). We then defined the time course of changed MeCP2 phosphorylation after cocaine. Finally, we evaluated the role of *N*-methyl-D-aspartate (NMDA) glutamate receptors in mediating the cocaine effect on MeCP2 expression and phosphorylation. In most experiments, a well-known transcriptional factor, CREB (cAMP response element-binding), was studied as a positive control.

2. Experimental procedures

2.1. Animals

Adult male Wistar rats weighing 225–275 g (Charles River, New York, NY) were individually housed in a controlled environment at a constant temperature of 23°C and humidity of 50 ± 10% with food and water available *ad libitum*. The animal room was on a 12-h/12-h light/dark cycle. Rats were allowed 6–7 days of habituation to the animal colony. All animal use and procedures were in strict accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.2. Systemic drug injection

Rats were subject to an intraperitoneal (i.p.) injection of saline or cocaine hydrochloride (Sigma-Aldrich, St. Louis, MO). The dose of cocaine was calculated as the salt. The dose of cocaine (30 mg/kg) was chosen based on the fact that the drug at this dose caused typical motor stimulation and significant increases in CREB phosphorylation in the striatum (Lesscher et al., 2005; Sun et al., 2007). Age-matched rats received an acute injection of saline (1 ml/kg) and served as controls. In pharmacological experiments evaluating the role of NMDA receptors, a competitive and systemically active NMDA receptor antagonist, (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), was used. CPP (Sigma) was dissolved in physiological saline and was injected at 10 mg/kg (i.p.) 30 min prior to cocaine (30 mg/kg, i.p.).

2.3. Protein extraction

Rats were anesthetized with Equithesin (9 ml/kg, i.p.) and decapitated at time points indicated. Brains were quickly removed and cut into coronal sections. The CPU, NAc, and mPFC were separately dissected into a 1.5 ml microtube containing RIPA buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1% SDS, a protease inhibitor cocktail (Thermo Scientific), and a phosphatase inhibitor cocktail (Thermo Scientific). The sample was homogenized by sonication. Protein concentrations in the whole tissue homogenate were determined and adjusted for Western blot analysis.

2.4. Western blot analysis

Western blot was performed as described previously (Yang et al., 2006; Zhang et al., 2007). Briefly, the equal amount of protein was separated on SDS NuPAGE Novex 4–12% gels (Invitrogen, Carlsbad, CA). Proteins were transferred to the polyvinylidene fluoride membrane (Millipore, Bedford, MA) and blocked in blocking buffer (5% nonfat dry milk in phosphate-buffered saline and 0.1% Tween 20) for 1 h. The blots were washed and incubated in the blocking buffer containing a primary rabbit antibody against phosphorylated MeCP2 (pMeCP2) at S421 (Abgent, San Diego, CA), MeCP2 (Abcam, Cambridge, MA), phosphorylated CREB (pCREB) at S133 (Santa Cruz Biotechnology, Santa Cruz, CA), CREB (Santa Cruz), or actin (Santa Cruz) usually at 1:1000 overnight at 4°C. This was followed by 1 h incubation in a horseradish peroxidase-linked secondary antibody against rabbit (Jackson ImmunoResearch Laboratory, West Grove, PA) at 1:5000. Immunoblots were developed with the enhanced chemiluminescence reagents (ECL; Amersham Pharmacia Biotech, Piscataway, NJ), and captured into Kodak Image Station 2000R. Kaleidoscope-prestained standards (Bio-Rad, Hercules, CA) and MagicMark XP Western protein standards (Invitrogen) were used for protein size determination. The density of immunoblots was measured using the Kodak 1D Image Analysis software (Liu et al., 2009; Mao et al., 2009).

2.5. Dephosphorylation assays

The specificity of pMeCP2 antibody was assessed in dephosphorylation assays. The striatum was dissected from brains removed from anesthetized rats. The dissected striatum was homogenized in a buffer containing 50 mM HEPES, pH 7.5, 150 mM NaCl, and 0.5% Triton X-100. The homogenate was treated with the protein phosphatase, λ -phosphatase (Santa Cruz; cat. no.: sc-200312), to dephosphorylate proteins. To this end, 80 μ l homogenates (4 μ g/ μ l) were treated with 20 μ l 5X phosphatase buffer (final concentration: 50 mM HEPES, pH 7.5, 0.1 mM EGTA, 5 mM dithiothreitol, 0.01% BRIJ 35, and 2 mM MnCl_2) containing 400 units of λ -phosphatase for 1.5 h at 30°C. Dephosphorylation reaction was terminated by adding 33 μ l 4X LDS sample loading buffer. Control samples (80 μ l) were treated with only 20 μ l 5X phosphatase buffer without λ -phosphatase for 1.5 h at 30°C, followed by addition of 33 μ l 4X LDS. Both control and dephosphorylated samples were boiled and subject to SDS-PAGE analysis (48 μ g/well) and probed with pMeCP2 or MeCP2 antibodies.

2.6. Statistics

The results are presented as means $\pm$ S.E.M., and were evaluated using a one-way analysis of variance followed by a Bonferroni (Dunn) comparison of groups using least squares-adjusted means or two-tailed unpaired Student's t-test. Probability levels of < 0.05 were considered statistically significant.

3. RESULTS

3.1. Specificity of antibodies

We first evaluated the specificity of the phospho- and site-specific antibody against pMeCP2 at S421. In Western blot analysis with striatal whole-cell homogenates, we detected a strong band around 75 kDa (Fig. 1A), similar to the appropriate molecular weight of pMeCP2-S421 observed from rat and mouse brain whole-cell extracts or cultured rat cortical neurons (Zhou et al., 2006; Tao et al., 2009). This band almost disappeared when striatal homogenates were subjected to a dephosphorylation treatment with λ -phosphatase (Fig. 1A). The antibody against total MeCP2 (tMeCP2) proteins recognized a band at a position corresponding to that of phosphorylated species of MeCP2 (Fig. 1B). These data support the specificity of the anti-pMeCP2-S421 antibody.

3.2. Effects of cocaine on MeCP2 and CREB phosphorylation in three forebrain regions

MeCP2 phosphorylation at S421 reduces its binding to methylated DNA and thus releases its repressor function over DNA transcription (Chen et al., 2003; Zhou et al., 2006). To determine the effect of cocaine on MeCP2 S421 phosphorylation, we subjected rats to a single dose of cocaine (30 mg/kg, i.p.). We sacrificed rats 20 min after drug injection to examine changes in S421-phosphorylated MeCP2 protein levels in the CPu, NAc, and mPFC. Selection of a 20-min time point was based on the fact that acute psychostimulants induced a peak increase in CERB phosphorylation in the rat striatum around this time point (Konradi et al., 1994; Simpson et al., 1995). We found that cocaine induced a slight increase in MeCP2 phosphorylation in the CPu ($117.8 \pm 3.8\%$ of saline), although it did not reach a statistically significant level ($P > 0.05$) (Fig. 2A). Cocaine did not affect total MeCP2 expression in this region (Fig. 2A). Acute cocaine is well known to induce a rapid increase in CREB phosphorylation in the CPu. We therefore tested the effect of cocaine on CREB phosphorylation in this region as a positive control. In fact, cocaine induced a substantial increase in CREB phosphorylation in the CPu ($436.7 \pm 78.6\%$ of saline, $P < 0.05$), while it had no effect on cellular levels of total CREB (tCREB) (Fig. 2B). These results indicate that

cocaine at a dose effective for stimulating CREB phosphorylation has no significant impact on MeCP2 phosphorylation in the CPu at a 20-min time point.

We then investigated the NAc for the response of MeCP2 phosphorylation to cocaine. Using NAc homogenates from the same rats, we found a significant elevation of pMeCP2 protein levels following cocaine treatment ($131.7 \pm 8.6\%$ of saline, $P < 0.05$) (Fig. 3A). There was no significant change in total MeCP2 levels in cocaine-treated animals compared to saline-treated rats (Fig. 3A). Thus, increased pMeCP2 levels result from an enhanced portion of phosphorylated MeCP2 in the total amount of MeCP2 proteins. Similar to the CPu, cocaine induced a marked increase in pCREB but not CREB levels in the NAc (Fig. 3B).

The mPFC is another primary site of dopamine projection and is an important structure of the brain reward system essentially implicated in the psychostimulant action (Tzschentke, 2000; Steketee, 2003). We therefore investigated whether MeCP2 phosphorylation in this area is subject to the modulation by cocaine. Cocaine did not cause a significant change in protein levels of pMeCP2 ($94.9 \pm 5.3\%$, $P > 0.05$) and MeCP2 (Fig. 4A). Only CREB phosphorylation was increased in cocaine-treated rats relative to saline-treated rats (Fig. 4B).

3.3. Time course of cocaine-regulated MeCP2 phosphorylation

To determine the temporal property of the cocaine-regulated MeCP2 phosphorylation, we subjected rats to acute cocaine (30 mg/kg). We then sacrificed animals at different time points (20, 60, and 180 min after drug injection) to assess changes in MeCP2 phosphorylation in the CPu and NAc. In these experiments, CREB phosphorylation was examined as a comparison. In the CPu, cocaine again had no significant effect on MeCP2 S421 phosphorylation at 20 min after injection ($115.1 \pm 6.8\%$ of saline, $P > 0.05$) (Fig. 5A). Interestingly, at 60 min, cocaine markedly elevated pMeCP2 levels ($147.1 \pm 7.0\%$ of saline, $P < 0.05$). This elevation declined at 180 min ($113.1 \pm 5.9\%$ of saline, $P > 0.05$). In contrast to MeCP2, CREB phosphorylation was considerably increased at an early time point (20 min) in cocaine-treated rats compared to saline-treated rats ($328.6 \pm 67.3\%$ of saline, $P < 0.05$) (Fig. 5B). This effect was typically transient as it rapidly dissipated at 60 min ($89.1 \pm 8.6\%$ of saline, $P > 0.05$). In the NAc, cocaine increased MeCP2 phosphorylation at an early 20-min time point ($137.4 \pm 9.0\%$ of saline, $P < 0.05$) (Fig. 6A). This significant increase persisted up to 60 min ($122.2 \pm 4.0\%$ of saline, $P < 0.05$), and declined at 180 min ($107.1 \pm 6.8\%$ of saline, $P > 0.05$). The cocaine-stimulated CREB phosphorylation in the NAc showed a transient pattern (Fig. 6B), similar to that seen in the CPu. There was no change in total protein expression for the two proteins in both the CPu and NAc structures at all time points surveyed (Fig. 5 and Fig. 6). These data reveal a relatively early and delayed increase in MeCP2 phosphorylation in the NAc and CPu, respectively, in response to cocaine stimulation.

3.4. Effects of CPP on cocaine-stimulated MeCP2 phosphorylation

Glutamate is a major neurotransmitter in the basal ganglia. NMDA glutamate receptors are densely expressed in striatal neurons (Landwehrmeyer et al., 1995; Chen and Reiner, 1996; Standaert et al., 1999; Kuppenbender et al., 2000). These receptors are implicated in psychostimulant-stimulated striatal gene expression (Torres and Rivier, 1993; Hanson et al., 1995; Konradi et al., 1996). A single high dose of cocaine increased extracellular levels of glutamate in the striatum (Smith et al., 1995; Reid et al., 1997). Enhanced glutamatergic transmission in the local striatum may then participate in mediating the effect of cocaine. To determine the relative importance of NMDA receptors in cocaine-stimulated MeCP2 phosphorylation, we investigated the effect of the NMDA receptor antagonist CPP on the ability of cocaine to induce MeCP2 phosphorylation in both the CPu and NAc. An i.p. injection of CPP at 10 mg/kg, a dose that blocked *zif/268* and preprodynorphin mRNA

expression in the rat striatum induced by acute amphetamine or methamphetamine (Wang and McGinty, 1994; 1996), had a minimal effect on basal MeCP2 phosphorylation in the CPu (Fig. 7A) and NAc (Fig. 7B). CPP injected 30 min prior to cocaine (30 mg/kg, 1 h) attenuated the cocaine-stimulated MeCP2 phosphorylation in the CPu (Fig. 7A). However, CPP failed to significantly attenuate the cocaine-stimulated MeCP2 phosphorylation in the NAc (Fig. 7B). These data suggest that activation of NMDA receptors in the CPu, although not in the NAc, is required for cocaine to stimulate MeCP2 phosphorylation.

4. Discussion

This study investigated the possible impact of cocaine on MeCP2 phosphorylation in several key regions of rat reward circuits. We found that acute injection of cocaine at a behaviorally active dose increased MeCP2 phosphorylation in both the CPu and NAc, while cocaine did not alter MeCP2 phosphorylation in the mPFC. The cocaine-stimulated MeCP2 phosphorylation in the two regions showed different kinetics. A relatively rapid and delayed increase in MeCP2 phosphorylation was induced in the NAc and CPu, respectively. In addition, NMDA glutamate receptors contribute to the effect of cocaine on MeCP2 phosphorylation in the CPu. Together, our results demonstrate that MeCP2 is a sensitive target of cocaine. Acute cocaine is able to increase its phosphorylation in striatal neurons *in vivo*. This event involves NMDA receptor activation in the CPu.

MeCP2 is expressed in most medium spiny neurons in the CPu and NAc (Deng et al., 2010). Acute injection of amphetamine (3 mg/kg) induced a robust increase in MeCP2 S421 phosphorylation in the mouse NAc, without increasing overall expression of MeCP2 (Deng et al., 2010). The increase was evident at 30 min and remained at 2 h after amphetamine injection. Acute cocaine (10 mg/kg) also increased S421 phosphorylation in the NAc (Deng et al., 2010), although detailed kinetics of this event was not analyzed. In this study, we extended these early findings to a time-course study. We found that cocaine generally like amphetamine induced a transient increase in MeCP2 phosphorylation in the NAc. Moreover, cocaine increased MeCP2 phosphorylation in the CPu. This increase showed a different time-course. The induced MeCP2 phosphorylation in the CPu seems to follow an early pMeCP2 induction in the NAc. Thus, the MeCP2 response to cocaine in its phosphorylation has a characteristic spatiotemporal pattern. The time- and region-dependent MeCP2 phosphorylation in the two striatal structures may imply distinct roles of MeCP2 in these regions in constructing a program of gene expression (see below) related to different phases and properties of cocaine action.

Acute amphetamine increased MeCP2 phosphorylation in the NAc through a signaling mechanism involving dopamine D1 receptors (Deng et al., 2010). In addition to dopamine signaling, glutamatergic transmission may participate in mediating the effect of cocaine. Dopamine and glutamate, two major neurotransmitters in the striatum, often synergize to regulate transcription factors, including CREB, in striatal neurons in response to psychostimulant stimulation (Konradi et al., 1996; Wang and McGinty, 1999). Blockade of either system is sufficient to reduce the effect of psychostimulants. In cultured hippocampal neurons, MeCP2 phosphorylation is positively regulated by a glutamate receptor-coupled Ca^{2+} signaling cascade, specifically NMDA receptor-mediated Ca^{2+} signals (Zhou et al., 2006). Thus, it is possible that responses of MeCP2 phosphorylation to cocaine in either of the CPu or NAc or both are sensitive to NMDA receptor signals. In this study, we found that MeCP2 responses in the CPu were blocked by an NMDA receptor antagonist, indicating that NMDA receptor activation is required for the upregulation of MeCP2 phosphorylation. However, MeCP2 responses in the NAc were insensitive to the NMDA receptor antagonist. This implies an insignificant role of NMDA receptors in this region. Why NMDA receptors play a different role in the two striatal regions is unclear. It may result from existing

differences in multiple profiles of glutamatergic transmission in these two regions, including glutamatergic innervations, subcellular/subsynaptic distributions of NMDA receptors, or interactions with dopamine and other transmitters. Of note, a single high dose of cocaine increased extracellular glutamate in the NAc (Smith et al., 1995; Reid et al., 1997). A lower dose of cocaine challenge also sufficiently increased glutamate in the NAc of cocaine-sensitized rats (Pierce et al., 1996; Reid and Berger, 1996; Bell et al., 2000). Released glutamate still has the opportunity to interact with other glutamate receptors, such as α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors or metabotropic glutamate receptors, to affect MeCP2 phosphorylation.

Psychostimulants have been well documented to induce neuroadaptations through the regulation of gene expression (Chao and Nestler, 2004; McClung and Nestler, 2008). These transcription-dependent neuroadaptations constitute a core metaplasticity for enduring drug-craving and seeking. Recent studies suggest that epigenetic modifications, including DNA methylation and associated binding of epigenetic factors/transcriptional repressors to methylated DNA for the sake of transcriptional silencing, are involved in the regulation of gene expression in response to psychostimulant exposure (Cassel et al., 2006; Anier et al., 2010; Deng et al., 2010). In this regard, MeCP2 is an attractive epigenetic factor. This factor can be rapidly phosphorylated at S421 in response to changing neural activity. Such phosphorylation reduces the ability of MeCP2 to act as a transcriptional repressor. Thus, the phosphorylation status of MeCP2 can be measured to reflect surrogate changes in the functional state of MeCP2 *in vivo* (Chen et al., 2003; Zhou et al., 2006). Both amphetamine and cocaine positively regulate S421 phosphorylation in striatal neurons (Deng et al., 2010; this study). This indicates that MeCP2 activity as a repressor is suppressed, leading to facilitation of downstream gene expression. Among MeCP2-targeted genes identified so far is BDNF, a neurotrophic peptide that regulates synaptic plasticity and cocaine addiction (Mao et al., 2010; McGinty et al., 2010). It has been reported that S421 phosphorylation releases MeCP2 from BDNF promoter and facilitates BDNF transcription (Chen et al., 2003; Martinowich et al., 2003; Zhou et al., 2006). Thus, increased MeCP2 phosphorylation may in part contribute to BDNF expression in the striatum induced by acute cocaine (Zhang et al., 2002) and more likely by chronic cocaine (Grimm et al., 2003; Cassel et al., 2006; Im et al., 2010). Other MeCP2-targeted genes may include FosB and JunB which are subject to the MeCP2 regulation in response to acute amphetamine (Deng et al., 2010) and are implicated in transcription-dependent neuroadaptations related to the addictive properties of psychostimulants of abuse (Hope et al., 1992; Hiroi et al., 1997; Kelz et al., 1999). Future studies will need to identify other MeCP2-regulated genes and their roles in neural plasticity and drug addiction.

Acknowledgments

This work was supported by NIH grants R01DA10355 (JQW) and R01MH61469 (JQW) and a grant from Saint Luke's Hospital Foundation.

References

- Anier K, Malinovskaja K, Aonurm-Helm A, Zharkovsky A, Kalda A. DNA methylation regulates cocaine-induced behavioral sensitization in mice. *Neuropsychopharmacology*. 2010; 35:2450–2461. [PubMed: 20720536]
- Bell K, Duffy P, Kalivas PW. Context-specific enhancement of glutamate transmission by cocaine. *Neuropsychopharmacology*. 2000; 23:335–344. [PubMed: 10942857]
- Cassel S, Carouge D, Gensburger C, Anglard P, Burgun C, Dietrich JB, Aunis D, Zwiller J. Fluorepine and cocaine induce the epigenetic factors MeCP2 and MBD1 in adult rat brain. *Mol Pharmacol*. 2006; 70:487–492. [PubMed: 16670375]

- Chahrour M, Zoghbi HY. The story of Rett syndrome: from clinic to neurobiology. *Neuron*. 2007; 56:422–437. [PubMed: 17988628]
- Chao HT, Zoghbi HY, Rosenmund C. MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. *Neuron*. 2007; 56:58–65. [PubMed: 17920015]
- Chao J, Nestler EJ. Molecular neurobiology of drug addiction. *Annu Rev Med*. 2004; 55:113–132. [PubMed: 14746512]
- Chen Q, Reiner A. Cellular distribution of the NMDA receptor NR2A/2B subunits in the rat striatum. *Brain Res*. 1996; 743:346–352. [PubMed: 9017267]
- Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, Jaenisch R, Greenberg ME. Depression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science*. 2003; 302:885–889. [PubMed: 14593183]
- Dani VS, Nelson SB. Intact long-term potentiation but reduced connectivity between neocortical layer 5 pyramidal neurons in a mouse model of Rett syndrome. *J Neurosci*. 2009; 29:11263–11270. [PubMed: 19741133]
- Deng JV, Rodriguiz RM, Hutchinson AN, Kim IH, Wetsel WC, West AE. MeCP2 in the nucleus accumbens contributes to neural and behavioral responses to psychostimulants. *Nat Neurosci*. 2010; 13:1128–1136. [PubMed: 20711186]
- Feng J, Nestler EJ. MeCP2 and drug addiction. *Nat Neurosci*. 2010; 13:1039–1041. [PubMed: 20740030]
- Gambino F, Khelfaoui M, Poulain B, Bienvenu T, Chelly J, Humeau Y. Synaptic maturation at cortical projections to the lateral amygdala in a mouse model of Rett syndrome. *PLoS One*. 2010; 5:e11399. [PubMed: 20625482]
- Gemelli T, Berton O, Nelson ED, Perrotti LI, Jaenisch R, Monteggia LM. Postnatal loss of methyl-CpG binding protein 2 in the forebrain is sufficient to mediate behavioral aspects of Rett syndrome in mice. *Biol Psychiatry*. 2006; 59:468–476. [PubMed: 16199017]
- Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y. Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. *J Neurosci*. 2003; 23:742–747. [PubMed: 12574402]
- Hanson GR, Singh N, Merchant K, Johnson M, Gibb JW. The role of NMDA receptor systems in neuropeptide responses to stimulants of abuse. *Drug Alcohol Depend*. 1995; 37:107–110. [PubMed: 7758400]
- Hiroi N, Marek GJ, Brown JR, Ye H, Saudou F, Vaidya VA, Duman RS, Greenberg ME, Nestler EJ. FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened sensitivity to cocaine's psychomotor and rewarding effects. *Proc Natl Acad Sci USA*. 1997; 94:10397–10402. [PubMed: 9294222]
- Hope B, Kosofsky B, Hyman SE, Nestler EJ. Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. *Proc Natl Acad Sci USA*. 1992; 89:5764–5768. [PubMed: 1631058]
- Im HI, Hollander JA, Bali P, Kenny PJ. MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nat Neurosci*. 2010; 13:1120–1127. [PubMed: 20711185]
- Kelz MB, Chen J, Carlezon WA Jr, Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang YJ, Marotti L, Self DW, Tkatch T, Baranaskas G, Surmeier DJ, Neve RL, Duman RS, Picciotto MR, Nestler EJ. Expression of the transcription factor Δ FosB in the brain controls sensitivity to cocaine. *Nature*. 1999; 401:272–276. [PubMed: 10499584]
- Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci*. 2006; 31:89–97. [PubMed: 16403636]
- Konradi C, Cole RL, Heckers S, Hyman SE. Amphetamine regulates gene expression in rat striatum via transcription factor CREB. *J Neurosci*. 1994; 14:5623–5634. [PubMed: 8083758]
- Konradi C, Levegue JC, Hyman SE. Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. *J Neurosci*. 1996; 16:4231–4239. [PubMed: 8753884]

- Kuppenbender KD, Standaert DG, Feuerstein TJ, Penney JB Jr, Young AB, Landwehrmeyer GB. Expression of NMDA receptor subunit mRNAs in neurochemically identified projection neurons and interneurons in the human striatum. *J Comp Neurol*. 2000; 419:407–421. [PubMed: 10742712]
- Landwehrmeyer GB, Standaert DG, Testa CM, Penney JB Jr, Young AB. NMDA receptor subunit mRNA expression by projection neurons and interneurons in rat striatum. *J Neurosci*. 1995; 15:5297–5307. [PubMed: 7623152]
- Lesscher HM, Hordijk M, Bondar NP, Alekseyenko OV, Burbach JP, van Ree JM, Gerrits MA. Mu-Opioid receptors are not involved in acute cocaine-induced locomotor activity nor in development of cocaine-induced behavioral sensitization in mice. *Neuropsychopharmacology*. 2005; 30:278–285. [PubMed: 15257307]
- Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F, Bird A. Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell*. 1992; 69:905–914. [PubMed: 1606614]
- Liu XY, Chu XP, Mao LM, Wang M, Lan HX, Li MH, Zhang GC, Parelkar NK, Fibuch EE, Haines M, Neve KA, Liu F, Xiong ZG, Wang JQ. Modulation of D2R-NR2B interactions in response to cocaine. *Neuron*. 2006; 52:897–909. [PubMed: 17145509]
- Mao LM, Fibuch EE, Wang JQ. Decoding BDNF-LTP coupling in cocaine addiction. *Neuron*. 2010; 67:679–681. [PubMed: 20890399]
- Mao LM, Wang W, Chu XP, Zhang GC, Liu XY, Yang YJ, Haines M, Papasian CJ, Fibuch EE, Buch S, Chen JG, Wang JQ. Stability of surface NMDA receptors controls synaptic and behavioral adaptations to amphetamine. *Nat Neurosci*. 2009; 12:602–610. [PubMed: 19349975]
- Martinowick K, Hattori D, Wu H, Fouse S, He F, Hu Y, Fan G, Sun YE. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science*. 2003; 302:890–893. [PubMed: 14593184]
- McClung CA, Nestler EJ. Neuroplasticity mediated by altered gene expression. *Neuropsychopharmacology*. 2008; 33:3–17. [PubMed: 17728700]
- McGinty JF, Whitfield TW Jr, Berglind WJ. Brain-derived neurotrophic factor and cocaine addiction. *Brain Res*. 2010; 1314:183–193. [PubMed: 19732758]
- Monteggia LM, Kavalali ET. Rett syndrome and the impact of MeCP2 associated transcriptional mechanisms on neurotransmission. *Biol Psychiatry*. 2009; 65:204–210. [PubMed: 19058783]
- Moretti P, Levenson JM, Battaglia F, Atkinson R, Teague R, Antalffy B, Armstrong D, Arancio O, Sweatt JD, Zoghbi HY. Learning and memory and synaptic plasticity are impaired in a mouse model of Rett syndrome. *J Neurosci*. 2006; 26:319–327. [PubMed: 16399702]
- Nelson ED, Kavalali ET, Monteggia LM. MeCP2-dependent transcriptional repression regulates excitatory neurotransmission. *Curr Biol*. 2006; 16:710–716. [PubMed: 16581518]
- Nelson ED, Kavalali ET, Monteggia LM. Activity-dependent suppression of miniature neurotransmission through the regulation of DNA methylation. *J Neurosci*. 2008; 28:395–406. [PubMed: 18184782]
- Pierce RC, Bell K, Duffy P, Kalivas PW. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *J Neurosci*. 1996; 16:1550–1560. [PubMed: 8778304]
- Reid MS, Berger SP. Evidence for sensitization of cocaine-induced nucleus accumbens glutamate release. *Neuroreport*. 1996; 7:1325–1329. [PubMed: 8817559]
- Reid MS, Hsu K Jr, Berger SP. Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. *Synapse*. 1997; 27:95–105. [PubMed: 9266771]
- Simpson JN, Wang JQ, McGinty JF. Repeated amphetamine administration induces a prolonged augmentation of phosphorylated cyclase response element-binding protein and Fos-related antigen immunoreactivity in rat striatum. *Neuroscience*. 1995; 69:441–457. [PubMed: 8552240]
- Smith JA, Mo Q, Guo H, Kunko PM, Robinson SE. Cocaine increases extraneuronal levels of aspartate and glutamate in the nucleus accumbens. *Brain Res*. 1995; 683:264–269. [PubMed: 7552364]

- Standaert DG, Friberg IK, Landwehrmeyer GB, Young AB, Penney JB Jr. Expression of NMDA glutamate receptors subunit mRNAs in Neurochemically identified projection and interneurons in the striatum of the rat. *Mol Brain Res*. 1999; 64:11–23. [PubMed: 9889300]
- Steketee JD. Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Res Rev*. 2003; 41:203–228. [PubMed: 12663081]
- Sun WL, Zhou L, Hazim R, Quinones-Jenab V, Jenab S. Effects of acute cocaine on ERK and DARPP-32 phosphorylation pathways in the caudate-putamen of Fischer rats. *Brain Res*. 2007; 1178:12–19. [PubMed: 17920048]
- Tao J, Hu K, Chang Q, Wu H, Sherman NE, Martinowich K, Klose RJ, Schanen C, Jaenisch R, Wang W, Sun YE. Phosphorylation of MeCP2 at Serine 80 regulates its chromatin association and neurological function. *Proc Natl Acad Sci USA*. 2009; 106:4882–4887. [PubMed: 19225110]
- Torres G, Rivier C. Cocaine-induced expression of striatal c-fos in the rat is inhibited by NMDA receptor antagonists. *Brain Res Bull*. 1993; 30:173–176. [PubMed: 8420627]
- Tzschentke TM. The medial prefrontal cortex as a part of the brain reward system. *Amino Acids*. 2000; 19:211–219. [PubMed: 11026491]
- Wang JQ, McGinty JF. NMDA receptors mediate amphetamine-induced upregulation of *zif/268* and preprodynorphin mRNA expression in rat striatum. *Synapse*. 1994; 18:343–353. [PubMed: 7886627]
- Wang JQ, McGinty JF. Acute methamphetamine-induced *zif/268*, preprodynorphin, and preproenkephalin mRNA expression in rat striatum depends on activation of NMDA and kainite/AMPA receptors. *Brain Res Bull*. 1996; 39:349–357. [PubMed: 9138744]
- Wang JQ, McGinty JF. Glutamate/dopamine interactions mediate the effects of psychostimulant drugs. *Addiction Biol*. 1999; 4:141–150.
- Yang L, Mao L, Chen H, Catavsan M, Kozinn J, Arora A, Liu X, Wang JQ. A signaling mechanism from Gαq-protein-coupled glutamate receptors to gene expression: role of the c-Jun N-terminal kinase pathway. *J Neurosci*. 2006; 26:971–980. [PubMed: 16421317]
- Zhang D, Zhang L, Lou DW, Nakabeppu Y, Zhang J, Xu M. The dopamine D1 receptor is a critical mediator for cocaine-induced gene expression. *J Neurochem*. 2002; 82:1453–1464. [PubMed: 12354293]
- Zhang GC, Mao LM, Liu XY, Parelkar NK, Arora A, Yang L, Haines M, Fibuch EE, Wang JQ. In vivo regulation of Homer1a expression in the striatum by cocaine. *Mol Pharmacol*. 2007; 71:1148–1158. [PubMed: 17234898]
- Zhou Z, Hong EJ, Cohen S, Zhao WN, Ho HY, Schmidt L, Chen WG, Lin Y, Savner E, Griffith EC, Hu L, Steen JA, Weitz CJ, Greenberg ME. Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. *Neuron*. 2006; 52:255–269. [PubMed: 17046689]

Highlights

- Cocaine is able to alter a critical epigenetic factor, methyl CpG-binding protein-2 (MeCP2), in selected brain regions related to reward and addiction.
- The effect of cocaine is rapid and transient.
- NMDA glutamate receptors may be involved in the effect of cocaine.
- MeCP2 may therefore link cocaine to drug addiction at the epigenetic level.

Figure 1. Evaluation of specificity of rabbit phospho- and site-specific antibodies

(A) A representative immunoblot with a rabbit anti-pMeCP2-S421 antibody. (B) A representative immunoblot with a rabbit anti-MeCP2 antibody. Note that strong bands of approximately 75 kDa (A) were detected from the control rat striatal homogenates but not from homogenates treated with λ -phosphatase (λ -PP). Striatal homogenates (80 μ l, 4 μ g/ μ l) were treated with λ -phosphatase (400 units) for 90 min at 30°C, followed by SDS-PAGE analysis.

Figure 2. Effects of cocaine on phosphorylation of MeCP2 and CREB in the rat CPu
(A) Effects of cocaine on MeCP2 S421 phosphorylation in the CPu. **(B)** Effects of cocaine on CREB S133 phosphorylation in the CPu. Representative immunoblots are shown left to the quantified data. Note that cocaine did not significantly increase MeCP2 phosphorylation, while it markedly increased CREB phosphorylation. Rats received a single dose of cocaine (30 mg/kg, i.p.) or saline and sacrificed 20 min after drug injection for immunoblot analysis. Data are presented as means $\pm$ SEM ($n = 4-7$ per group). * $P < 0.05$ versus saline.

Figure 3. Effects of cocaine on phosphorylation of MeCP2 and CREB in the rat NAc
(A) Effects of cocaine on MeCP2 S421 phosphorylation in the NAc. **(B)** Effects of cocaine on CREB S133 phosphorylation in the NAc. Representative immunoblots are shown left to the quantified data. Note that cocaine significantly increased MeCP2 phosphorylation, while it did not affect total MeCP2 expression. Rats received a single dose of cocaine (30 mg/kg, i.p.) or saline and sacrificed 20 min after drug injection for immunoblot analysis. Data are presented as means $\pm$ SEM ($n = 4-7$ per group). * $P < 0.05$ versus saline.

Figure 4. Effects of cocaine on phosphorylation of MeCP2 and CREB in the rat mPFC
(A) Effects of cocaine on MeCP2 S421 phosphorylation in the mPFC. **(B)** Effects of cocaine on CREB S133 phosphorylation in the mPFC. Representative immunoblots are shown left to the quantified data. Rats received a single dose of cocaine (30 mg/kg, i.p.) or saline and sacrificed 20 min after drug injection for immunoblot analysis. Data are presented as means $\pm$ SEM (n = 4–7 per group). * P < 0.05 versus saline.

Figure 5. Time-dependent effects of cocaine on phosphorylation of MeCP2 and CREB in the rat CPu

(A) Time-dependent effects of cocaine on MeCP2 phosphorylation in the CPu. (B) Time-dependent effects of cocaine on CREB phosphorylation in the CPu. Representative immunoblots are shown left to the quantified data. Note that cocaine induced a delayed increase in MeCP2 phosphorylation relative to that in CREB phosphorylation. Rats received a single dose of cocaine (30 mg/kg, i.p.) or saline and sacrificed at different time points (20, 60, or 180 min after drug injection) for immunoblot analysis. Data are presented as means $\pm$ SEM ($n = 4-6$ per group). * $P < 0.05$ versus saline.

Figure 6. Time-dependent effects of cocaine on phosphorylation of MeCP2 and CREB in the rat NAc

(A) Time-dependent effects of cocaine on MeCP2 phosphorylation in the NAc. **(B)** Time-dependent effects of cocaine on CREB phosphorylation in the NAc. Representative immunoblots are shown left to the quantified data. Rats received a single dose of cocaine (30 mg/kg, i.p.) or saline and sacrificed at different time points (20, 60, or 180 min after drug injection) for immunoblot analysis. Data are presented as means $\pm$ SEM ($n = 4-6$ per group). * $P < 0.05$ versus saline.

Figure 7. Effects of CPP on cocaine-stimulated MeCP2 phosphorylation in the rat striatum
(A) Effects of CPP on cocaine-stimulated MeCP2 phosphorylation in the CPu. **(B)** Effects of CPP on cocaine-stimulated MeCP2 phosphorylation in the NAc. Representative immunoblots are shown above the quantified data. Note that CPP reduced cocaine-induced MeCP2 phosphorylation in the CPu but not NAc. Rats were treated with an i.p. injection of CPP (10 mg/kg) 30 min prior to an i.p. injection of cocaine (30 mg/kg). They were then sacrificed 60 min after cocaine injection for immunoblot analysis of MeCP2 phosphorylation. Data are presented as means $\pm$ SEM (N = 5 per group). * $P < 0.05$ versus saline + saline. + $P < 0.05$ versus saline + cocaine.