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c-Fos is an intracellular regulator of cocaine-induced long-term changes

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Abstract

Development of drug addiction is accompanied by the induction of long-lasting neurobiological changes. Dopamine D1 receptors are involved in mediating cocaine-induced neuroadaptation yet the underlying intracellular mechanisms remain less clear. Using a genetically modified mouse in which *Fos* is primarily mutated in D1 receptor-bearing neurons in the brain, we examined a potential role of the immediate early gene *Fos*, which is rapidly induced by cocaine via D1 receptors, in mediating cocaine-induced persistent neurobiological changes. We found that the composition of AP-1 transcription complexes and expression levels of AP-1 complexes, and several transcription factors, neurotransmitter receptors as well as intracellular signaling molecules following repeated cocaine administration are altered in *Fos*-deficient brains. Moreover, dendritic reorganization of medium spiny neurons induced by repeated exposure to cocaine is attenuated in the mutant brains. The mutant mice also exhibit reduced behavioral sensitization following repeated cocaine administration. These findings suggest that c-Fos expressed in D1 receptor-bearing neurons mediates cocaine-induced persistent changes.

Keywords

cocaine; dopamine D1 receptors; signaling; c-Fos; gene expression; dendritic morphology; behaviors

INTRODUCTION

Drug addiction is a brain disease that it is long-lasting.1–9 The dopamine (DA) pathways that project from the midbrain to the nucleus accumbens (NAc), caudoputamen (CPu), amygdala (AMG), hippocampus (HIP) and prefrontal cortex (PFC) play a key part in mediating the enduring effects of drugs of abuse.10–11 Abused drugs increase synaptic levels of DA that mediate reward and reinforcement.12–14

DA D1 receptors are expressed in the NAc, CPu, AMG, HIP and PFC,15 brain regions that mediate the neurobiological effects of cocaine and other drugs of abuse.10–14 Some pharmacological treatments for cocaine addicts have targeted D1 receptors.16–19 This is because D1 receptor agonists and antagonists can influence cocaine-induced locomotor responses, discriminative stimulus, reinforcing effects and reinstatement in a variety of experimental paradigms.20–30 Moreover, repeated exposure to cocaine leads to persistent increases in D1 receptor-mediated inhibitory responses of NAc neurons and reorganization of brain circuits involving D1 receptor-expressing neurons.31–32 Using D1 receptor mutant mice, we have demonstrated that this receptor is necessary in mediating cocaine-induced

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behavioral, electrophysiological, dendritic morphological, cell signaling and gene expression changes.[33–39] Together, these results support the view that the D1 receptor is a key cell surface receptor mediating the neurobiological effects of cocaine.

The enduring nature of drug addiction suggests that repeated exposure to drugs leads to stable alterations that involve persistent changes in neuronal circuits, cell signaling and gene expression.[3–9] Some of these alterations are mediated by transcription factors ΔFosB and the cAMP-response element binding protein (CREB) via regulation of gene expression.[3–4,9,40–43] The complexity of neuroadaptations and the multi-faceted nature of behavioral changes induced by repeated exposure to cocaine suggest the involvement of additional Fos family proteins and their target genes.[44] The most prominent molecular response to acute D1 receptor agonists or cocaine stimulations is a transient up-regulation of the immediate early gene (IEG) *Fos* expression in the NAc, CPu, AMG and PFC.[3,42,45–46] c-Fos induction by cocaine is dependent on D1 receptors.[47–50] Repeated exposure to cocaine leads to a gradual decease in *Fos* inducibility.[32,46] The c-Fos proteins form heterodimers with Jun family proteins resulting in AP-1 transcription complex formation and regulation of cellular gene expression.[51] These evidence suggests that c-Fos may mediate neurobiological responses to cocaine by regulating changes in the level and composition of AP-1 complexes and patterns of gene expression in D1 receptor-bearing neurons.[36] To test this notion, we generated a mouse in which *Fos* is selectively mutated in this group of neurons. Our findings suggest that c-Fos in D1 receptor-bearing neurons mediates long-lasting changes induced by repeated cocaine administration.[52]

METHODS AND METHODS

For details of the generation of the *Fos* mutant mice (*f/f-Fos-D1-Cre* mice) and subsequently analyses, see Zhang et al. and references therein.[52]

RESULTS

A mouse model carrying a D1 receptor-bearing neuron-specific *Fos* mutation

To investigate how c-Fos may mediate cocaine-induced persistent neurobiological changes, we used a *Cre/loxP*-mediated DNA deletion strategy to make a mouse with a selective *Fos* mutation in D1 receptor-bearing neurons.[52–54] We made several lines of *Cre* transgenic mice in which *Cre* expression is driven by an 8 kilobase pair promoter of the D1 receptor gene that has been shown to be sufficient to drive normal D1 receptor expression in transgenic mice.[52,55] Extensive analysis of crosses between varies lines of *D1-Cre* transgenic mice and an indicator mouse identified a line of mouse in which the Cre activity resided in neurons in all major regions of normal D1 receptor expression, including the shell and core of the NAc, CPu, AMG, HIP and PFC starting at 3 weeks of age and reaching peak levels at 7 weeks of age.[52] Importantly, the Cre activity is absent in this line of mouse between 7 to 28 weeks of age in brain regions where the D1 receptor gene is not highly expressed, such as the cerebellum.[52]

After crossing this line of *D1-Cre* mouse with an *f/f-Fos* mouse, we performed immunostaining for c-Fos. The acute c-Fos induction by a D1 receptor agonist SKF81297 or by cocaine was greatly reduced in the CPu and NAc of *f/f-Fos-D1-Cre* mice 7 weeks of age or older compared to that in wild-type mice. We also performed co-immunostaining for c-Fos and dynorphin which co-localizes significantly with the D1 receptor particularly in the dorsal striatum. In the NAc and CPu, dynorphin expression was qualitatively not affected by the *Fos* mutation. By contrast, while dynorphin and c-Fos were co-expressed in striatal neurons in wild-type mice, little c-Fos was induced by acute cocaine administration in

dynorphin-positive striatal neurons. These results demonstrate that *Fos* expression is largely eliminated in D1 receptor-bearing neurons in *f/f-Fos-D1-Cre* mice.52

To examine further whether the *Fos* mutation is largely limited to D1 receptor-bearing neurons, we treated *f/f-Fos-D1-Cre* and wild-type mice between 7 to 28 weeks of age with a D2 class receptor antagonist haloperidol that can induce *Fos* expression in the CPu. Both groups of mice exhibited similar cataleptic behaviors following haloperidol injections. Importantly, *Fos* was induced by haloperidol in the CPu in both groups of mice, with mutant mice exhibiting 8% and 9% less c-Fos signals compared to the wild-type mice at the 0.5 and 2 mg/kg haloperidol doses, respectively. Kainic acid, which elicits widespread c-Fos expression in the brain, induced similar levels of c-Fos in brain regions where the D1 receptor is poorly or not expressed, including the cingulate cortex, entorhinal cortex, median eminence and VTA in the two groups of mice. These results indicate that the *Fos* mutation is primarily limited to D1 receptor-bearing neurons in the age range we used.52

f/f-Fos-D1-Cre mice appear healthy with no obvious abnormalities in the brain. Acute locomotor responses induced by two different doses of D1 or D2 receptor agonists were similar between wild-type and *f/f-Fos-D1-Cre* mice.52

c-Fos regulates IEG induction and AP-1 transcription complex formation in the NAc and CPu after repeated cocaine administration

To test the notion that c-Fos-regulated gene expression may mediate cocaine-induced long-lasting changes, we investigated how c-Fos regulates the expression of other IEGs before and after cocaine administration. There was no baseline difference in IEG expression in the NAc and CPu in the two groups of mice, suggesting that the *Fos* mutation did not lead to obvious compensatory changes by other Fos family proteins in D1 receptor-bearing neurons. An acute cocaine injection raised FosB and Fra-2 levels by 2 and 5 fold, respectively, in the NAc and CPu in wild-type mice, while such induction failed in *f/f-Fos-D1-Cre* mice. Following 7 days of cocaine administration, FosB and ΔFosB levels were increased by 1.5 and 3 fold, respectively, in wild-type mice whereas they were 30% and 65% lower, respectively, in the NAc and CPu in *f/f-Fos-D1-Cre* mice compared to that in wild-type mice.52 The expression of other IEGs was similar in the two groups of mice.52

The lack of *Fos* and the subsequent changes in FosB and ΔFosB expression may affect the level and composition of the AP-1 complexes in *f/f-Fos-D1-Cre* mice compared to those in wild-type mice before and after exposure to cocaine. Band-shift experiment showed that, whereas baseline levels are similar, *f/f-Fos-D1-Cre* mice exhibit 40% less AP-1 transcription complexes compared to wild-type mice after acute cocaine injections. Supershift experiment showed that there are less FosB/ΔFosB in AP-1 complexes in the NAc and CPu in *f/f-Fos-D1-Cre* mice compared to that in wild-type mice after both acute (0.2:1 versus 0.8:1) and repeated (1:1 versus 4:1) cocaine injections.52 These results demonstrate that a lack of *Fos* in D1 receptor-bearing neurons affects FosB and ΔFosB levels and AP-1 transcription complex formation in the NAc and CPu following cocaine treatment.

c-Fos regulates additional target gene expression in the NAc and CPu following repeated cocaine administration

The changed AP-1 transcription complexes due to the *Fos* deficiency may affect target gene expression in the NAc and CPu before and after exposure to cocaine. To test this notion, we investigated the expression of two classes of genes that contain AP-1 binding sites in their promoter regions. The first class encodes glutamate receptors. Blockade of the *N*-methyl-*D*-aspartate receptor 1 subunit (NR1) attenuates stimulant-induced behavioral sensitization.56 Persistent ΔFosB expression or repeated cocaine injections induce the α-amino-3-

hydroxy-5-methyl-4-isoxazole propionic acid receptor subunit 2 (GluR2) expression in the NAc,41 and extinction of cocaine self-administration up-regulates GluR2 and reduces cocaine-seeking behaviors.57 Western blotting showed that 7 or 28 days of repeated cocaine injections led to a 1.3–1.5 fold increase in NR1 and GluR2 expression in the NAc and CPu of wild-type mice and failed to do so in *f/f-Fos-D1-Cre* mice.52 Basal expression of the two genes was identical in the two groups of mice.52 The expression of NR2A, NR2B and GluR1 was not obviously different in the two groups of mice before and after cocaine administration.

The second class of genes encodes signaling molecules β-catenin, Cdk5 and p35. β-catenin is involved in intercellular junctions and in the Wnt signaling pathway, and it can be induced by cocaine via D1 receptors in the NAc and CPu.49 Cdk5 is critical in neurite outgrowth and in cocaine-induced behavior and dendritic reorganization in the NAc.58 Cdk5 activity is regulated by p35.59 Repeated cocaine administration increased the expression of β-catenin and p35 by 1.3–1.7 fold in the NAc and the CPu of wild-type mice while such induction was attenuated in *f/f-Fos-D1-Cre* mice. Cdk5 expression was different by 1.3 fold in the two groups of mice after 28 days but not 7 days of cocaine administration.52 Basal expression of these three genes was similar in the two groups of mice.

Consistent with those observed *in vivo*, increasing levels of c-Fos increased the expression of *Grin1*, *Gria2*, *Ctnnb* and *Cdk5r1 in vitro*.52 These findings show that c-Fos regulates the expression of target genes both *in vivo* and *in vitro*.

c-Fos mediates dendritic reorganization of medium spiny neuron induced by repeated cocaine administration

Repeated cocaine injections alter the number of dendrites and the density of dendritic spines of neurons in the NAc and cortex.60–61 This dendritic reorganization may reflect changes in neuronal circuits and contribute to the persistence of cocaine-induced behaviors. We investigated whether *Fos* mediates basal and cocaine-induced neuronal morphological changes by treating *f/f-Fos-D1-Cre* and wild-type mice with saline or cocaine for 28 days and performing dendritic morphological analysis. There was an increase in dendritic branching on medium spiny neurons in the NAc shell and CPu following cocaine injections compared to that after saline injections in wild-type mice but not in *f/f-Fos-D1-Cre* mice. Repeated cocaine injections also led to an increase in dendritic spine density on medium spiny neurons in the NAc shell and CPu compared to saline injections in wild-type mice, yet again not in *f/f-Fos-D1-Cre* mice. Whereas there was no obvious baseline difference, the number of dendrites and density of dendritic spines of neurons in the NAc shell and CPu were significantly different between the two groups of mice following repeated cocaine administration.52 These results suggest that *f/f-Fos-D1-Cre* mice exhibit less dendritic reorganization than wild-type mice in response to repeated cocaine administration.

c-Fos contributes to behavioral sensitization to cocaine

Changes in gene expression and dendritic reorganization induced by repeated cocaine administration may contribute to long-lasting behavioral changes such as behavioral sensitization.62–63 We investigated the potential contribution of c-Fos in this behavioral change by treating *f/f-Fos-D1-Cre* and wild-type mice either acutely or once daily cocaine for 7 days and again after 72 hours. While there were no differences in baseline activity after saline injections between mutant mice and wild-type mice, acute cocaine administration at the 20 mg/kg dose induced significant locomotion compared to saline injections and repeated cocaine injections at both the 10 and 20 mg/kg doses induced behavioral sensitization in wild-type yet not in *f/f-Fos-D1-Cre* mice. Repeated saline injections did not induce noticeable changes in locomotion in wild-type and *f/f-Fos-D1-Cre* mice. These

results suggest that c-Fos contributes to both the acute locomotor activation and the development of behavioral sensitization induced by cocaine in the injection paradigms we used.

Wild-type and *f/f-Fos-D1-Cre* mice did not show basal differences in rearing and grooming. Acute cocaine treatment at the 20 mg/kg dose induced rearing in both groups of mice with wild-type mice exhibiting more rearing than *f/f-Fos-D1-Cre* mice. In contrast, grooming behavior was not induced by acute cocaine injections in either group of mice. After repeated cocaine injections at the 20 mg/kg dose, wild-type but not *f/f-Fos-D1-Cre* mice developed sensitized rearing compared to that after the first cocaine injection. Both wild-type and *f/f-Fos-D1-Cre* mice developed sensitized grooming behaviors and there was no difference between the two groups of mice. At this cocaine dose, we did not observe additional stereotyped behaviors.

DISCUSSION

Repeated exposure to cocaine induces long-lasting changes in behavior, neuronal circuits and gene expression via DA D1 receptors. Although c-Fos is a transcription factor that is robustly and rapidly induced by D1 receptor agonists and cocaine in D1 receptor-bearing neurons, whether such induction plays a major role in modulating any neuronal responses to D1 receptor activation is unknown. By generating and analyzing a mouse in which *Fos* is primarily mutated in D1 receptor-bearing neurons, we investigated this issue in the context of cocaine-induced persistent changes. Our results suggest that c-Fos is an intracellular regulator of cocaine-induced alteration in gene expression, reorganization of neuronal circuits, and the development and manifestation of behavioral sensitization.

The D1 receptor neuron-specific *Fos* mutant mouse model

We engineered a mouse carrying a *Fos* mutation in D1 receptor-bearing neurons. The D1 receptor agonist SKF81297- and cocaine-induced c-Fos expression is significantly reduced in the CPu and NAc in *f/f-Fos-D1-Cre* mice compared to that in wild-type mice. Moreover, whereas dynorphin expression is qualitatively unaffected, cocaine-induced *Fos* expression is significantly reduced in dynorphin-positive neurons in the CPu and NAc in *f/f-Fos-D1-Cre* mice compared to those in wild-type mice. Further, haloperidol induced rich c-Fos expression in the CPu in *f/f-Fos-D1-Cre* and wild-type mice. Kainic acid-induced *Fos* expression is similar in the two groups of mice in brain areas that express D1 receptors poorly. These lines of evidence indicate that the *Fos* mutation is primarily limited to D1 receptor-bearing neurons in *f/f-Fos-D1-Cre* mice within the 7–28 week age range.

Unlike D1 receptor mutant mice,33¯34 *f/f-Fos-D1-Cre* mice exhibit apparent normal development with similar baseline motor activity, acute locomotor responses to SKF81297, quinpirole and haloperidol treatment, and baseline dendritic structures and dendritic spine density compared to wild-type mice. There are no obvious abnormalities in the brains of *f/f-Fos-D1-Cre* mice. Basal IEG expression levels and basal and cocaine-induced expression of dynorphin are also similar in the two groups of mice. These results suggest that the *Fos* mutation did not drastically affect the development of the DA system in *f/f-Fos-D1-Cre* mice.

c-Fos mediates cocaine-induced dendritic reorganization

Drug-induced dendritic reorganization may reflect changes in neuronal circuits including synaptic strength and connections. We found that the *Fos* deficiency in D1 receptor-bearing neurons results in attenuation in chronic cocaine-induced dendritic remodeling in the NAc and CPu in *f/f-Fos-D1-Cre* mice without affecting basal dendritic morphology. This result

suggests that it is the repeated transient up-regulation of c-Fos by cocaine rather than basal c-Fos that is involved in dendritic reorganization. The lack of significant dendritic remodeling in *f/f-Fos-D1-Cre* mice is similar to that of the D1 receptor mutant mice following repeated cocaine administration, suggesting that these two molecules may be in the same pathway in mediating cocaine-induced dendritic remodeling. Inhibiting Cdk5 activity can attenuate cocaine-induced increase in dendritic spine density.61 We found that c-Fos up-regulates p35 and Cdk5 levels in the NAc and CPu following 28 days of cocaine injections and the time course for such regulation correlates with dendritic remodeling. Although Cdk5 levels are not directly affected by changes in the levels of c-Fos after 7 days of cocaine injections, changed p35 levels may well change Cdk5 activity. These findings suggest that, upon repeated exposure to cocaine, c-Fos expressed in D1 receptor-bearing neurons mediates signaling events leading to dendritic reorganization that may contribute to the persistence of drug addiction.

c-Fos contributes to cocaine-induced behavioral sensitization

Behavioral sensitization is thought to be similar to the intensification of drug craving after repeated exposure, and is thought to be involved in drug-seeking behaviors in humans. We previously found that the D1 receptor is necessary for the induction of behavioral sensitization by cocaine.35 In the current study, we found that *f/f-Fos-D1-Cre* mice exhibit attenuated behavioral sensitization to cocaine compared to wild-type mice. *f/f-Fos-D1-Cre* mice also exhibit less sensitized responses in rearing and comparable grooming compared to wild-type mice after repeated cocaine treatment. These results suggest that c-Fos expressed in D1 receptor-bearing neurons likely contributes to the development of persistent behavioral changes induced by repeated exposure to cocaine.

c-Fos may mediate cocaine-induced persistent changes by regulating AP-1 transcription complex formation and gene expression

ΔFosB mediates several neurobiological effects of cocaine.3,41,43 We found that the c-Fos mutation leads to a reduced FosB expression following acute cocaine injections, and reduced FosB and ΔFosB expression after repeated cocaine administration in *f/f-Fos-D1-Cre* mice compared to wild-type mice. Moreover, less FosB/ΔFosB are in AP-1 transcription complexes in the NAc and CPu in *f/f-Fos-D1-Cre* mice compared to wild-type mice after either acute or repeated exposure to cocaine. These findings are similar to those found in D1 receptor mutant mice,49 suggesting that a possible mechanism for c-Fos to mediate cocaine-induced persistent changes is to regulate ΔFosB expression, and ΔFosB-regulated target genes, in D1 receptor-bearing neurons. Because c-Fos and ΔFosB have different half-lives, and c-Fos- and ΔFosB-containing AP-1 transcription complexes have different AP-1 binding affinities, c-Fos likely also regulates a set of target genes that is not regulated by ΔFosB following exposure to cocaine. We found that, regardless of their induction status, Fra-1, Fra-2, c-Jun, JunB and JunD all participate in AP-1 transcription complexes in the NAc and CPu and the extent of participation of each protein changes with more cocaine injections, suggesting that they may also play roles in cocaine-induced neuroadaptations.

Although not having a significant effect on basal levels, the c-Fos deficiency affected *Grin1*, *Gria2*, *Ctbnn* and *Cdk5r1* induction in the NAc and CPu *in vivo* by repeated cocaine administration, while over-expression of c-Fos up-regulated levels of these genes *in vitro*. These findings suggest that the attenuated induction of these genes in *f/f-Fos-D1-Cre* mice following repeated cocaine administration is likely due to a lack of direct transcriptional regulation by c-Fos rather than to potential indirect effects of the *Fos* mutation. Following repeated cocaine injections, the enhanced NR1 and GluR2 expression may change synaptic transmission, and the increased β-catenin and p35 expression may affect neuronal connections in the NAc and CPu.52

Our studies using *f/f-Fos-D1-Cre* mice reveal several neurobiological and behavioral phenotypes that parallel those found using D1 receptor mutant mice in the context of cocaine-induced neuroplastic changes. First, both D1 receptors and c-Fos expressed in D1 receptor-bearing neurons contribute to aspects of the locomotor stimulating effects of cocaine.[33–35,52] Second, both D1 receptors and c-Fos mediate dendritic reorganization and regulate gene expression induced by cocaine.[36–37,39,42,50,52] Third, the D1 receptor is required for c-Fos induction by cocaine and some of cocaine-regulated genes that are mediated by both D1 receptors and c-Fos.[36,46–50,52] These findings support a molecular model in which repeated cocaine administration results in repeated and gradually reduced transient up-regulation of c-Fos expression via D1 receptors. Changed levels of c-Fos, in turn, change the level and composition of AP-1 transcription complexes and regulate mechanisms within D1 receptor-bearing neurons to mediate cocaine-induced expression of cellular genes, reorganization of neuronal circuits, and the development of behavioral responses.

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