Review

Transcriptional mechanisms of addiction: role of ΔFosB

Eric J. Nestler*

Department of Neuroscience, Mount Sinai School of Medicine, New York, NY 10029, USA

Regulation of gene expression is considered a plausible mechanism of drug addiction, given the stability of behavioural abnormalities that define an addicted state. Among many transcription factors known to influence the addiction process, one of the best characterized is ΔFosB, which is induced in the brain’s reward regions by chronic exposure to virtually all drugs of abuse and mediates sensitized responses to drug exposure. Since ΔFosB is a highly stable protein, it represents a mechanism by which drugs produce lasting changes in gene expression long after the cessation of drug use. Studies are underway to explore the detailed molecular mechanisms by which ΔFosB regulates target genes and produces its behavioural effects. We are approaching this question using DNA expression arrays coupled with the analysis of chromatin remodelling—changes in the posttranslational modifications of histones at drug-regulated gene promoters—to identify genes that are regulated by drugs of abuse via the induction of ΔFosB and to gain insight into the detailed molecular mechanisms involved. Our findings establish chromatin remodelling as an important regulatory mechanism underlying drug-induced behavioural plasticity, and promise to reveal fundamentally new insight into how ΔFosB contributes to addiction by regulating the expression of specific target genes in brain reward pathways.

Keywords: chromatin remodelling; epigenetics; nucleus accumbens; orbitofrontal cortex; ventral tegmental area

Abbreviations: BNST, bed nucleus of the stria terminalis; IPAC, interstitial nucleus of the posterior limb of the anterior commissure; PAG, periaqueductal grey; VTA, ventral tegmental area; SN, substantia nigra

1. INTRODUCTION

The study of transcriptional mechanisms of addiction is based on the hypothesis that regulation of gene expression is one important mechanism by which chronic exposure to a drug of abuse causes long-lasting changes in the brain, which underlie the behavioural abnormalities that define a state of addiction (Nestler 2001). A corollary of this hypothesis is that drug-induced changes in dopaminergic and glutamatergic transmission and in the morphology of certain neuronal cell types in the brain, which have been correlated with an addicted state, are mediated in part via changes in gene expression.

Work over the past 15 years has provided increasing evidence for a role of gene expression in drug addiction, as several transcription factors—proteins that bind to specific response elements in the promoter regions of target genes and regulate those genes’ expression—have been implicated in drug action. Prominent examples include ΔFosB (a Fos family protein), cAMP-response element-binding protein (CREB), inducible cAMP early repressor (ICER), activating transcription factors (ATFs), early growth response proteins (EGRs), nucleus accumbens 1 (NAC1), nuclear factor κB (NFκB) and glucocorticoid receptor (O’Donovan et al. 1999; Mackler et al. 2000; Ang et al. 2001; Deroche-Gamonet et al. 2003; Carlezon et al. 2005; Green et al. 2006, 2008). This review focuses on ΔFosB, which appears to play a unique role in the addiction process, as a way to illustrate the types of experimental approaches that have been used to investigate transcriptional mechanisms of addiction.

2. INDUCTION OF ΔFosB IN NUCLEUS ACCUMBENS BY DRUGS OF ABUSE

ΔFosB is encoded by the fosB gene (figure 1) and shares homology with other Fos family transcription factors, which include c-Fos, FosB, Fra1 and Fra2 (Morgan & Curran 1995). These Fos family proteins heterodimerize with Jun family proteins (c-Jun, JunB or JunD) to form active activator protein-1 (AP-1) transcription factors that bind to AP-1 sites (consensus sequence: TGAC/GTCA) present in the promoters of certain genes to regulate their transcription. These Fos family proteins are induced rapidly and transiently in specific brain regions after acute administration of many drugs of abuse (figure 2; Graybiel et al. 1990; Young et al. 1991; Hope et al. 1992). These
responses are most prominently in nucleus accumbens and dorsal striatum, which are important mediators of the rewarding and locomotor actions of the drugs. All of these Fos family proteins, however, are highly unstable and return to basal levels within hours of drug administration.

Very different responses are seen after chronic administration of drugs of abuse (figure 2). Biochemically modified isoforms of ΔFosB (M, 35–37 kD) accumulate within the same brain regions after repeated drug exposure, whereas all other Fos family members show tolerance (i.e. reduced induction compared with initial drug exposures; Chen et al. 1995, 1997; Hiroi et al. 1997). Such accumulation of ΔFosB has been observed for virtually all drugs of abuse (table 1; Hope et al. 1994; Nye et al. 1995; Moratalla et al. 1996; Nye & Nestler 1996; Pich et al. 1997; Muller & Unterwald 2005; McDaids et al. 2006b), although different drugs differ somewhat in the relative degree of induction seen in nucleus accumbens core versus shell and dorsal striatum (Perrotti et al. 2008). At least for some drugs of abuse, the induction of ΔFosB appears selective for the dynorphin-containing subset of medium spiny neurons located in these brain regions (Nye et al. 1995; Moratalla et al. 1996; Muller & Unterwald 2005; Lee et al. 2006), although more work is needed to establish this with certainty. The 35–37 kD isoforms of ΔFosB dimerize predominantly with JunD to form an active and long-lasting AP-1 complex within these brain regions (Chen et al. 1997; Hiroi et al. 1998; Pérez-Otaño et al. 1998). The drug induction of ΔFosB in the nucleus accumbens seems to be a response to the pharmacological properties of the drug per se and not related to volitional drug intake, since animals that self-administer cocaine or receive yoked drug injections show equivalent induction of this transcription factor in this brain region (Perrotti et al. 2008).

The 35–37 kD ΔFosB isoforms accumulate with chronic drug exposure due to their extraordinarily long half-lives (Chen et al. 1997; Alibhai et al. 2007).

By contrast, there is no evidence that the splicing of ΔFosB or the stability of its mRNA is regulated by drug administration. As a result of its stability, therefore, the ΔFosB protein persists in neurons for at least several weeks after the cessation of drug exposure. We now know that this stability is due to the following two factors (figure 1): (i) the absence of two degron domains in ΔFosB, which are present at the C-terminus of full-length FosB and all other Fos family proteins and target those proteins to rapid degradation and (ii) the phosphorylation of ΔFosB at its N-terminus by casein kinase 2 and perhaps other protein kinases (Ulery et al. 2006; Carle et al. 2007). The stability of the ΔFosB isoforms provides a novel molecular mechanism by which drug-induced changes in gene expression can persist despite relatively long periods of drug withdrawal. We have, therefore, proposed that ΔFosB functions as a sustained ‘molecular switch’ that helps initiate and then maintain an addicted state (Nestler et al. 2001; McClung et al. 2004).

3. ROLE OF ΔFOSB IN NUCLEUS ACCUMBENS IN REGULATING BEHAVIOURAL RESPONSES TO DRUGS OF ABUSE

Insight into the role of ΔFosB in drug addiction has come largely from the study of bitransgenic mice in which ΔFosB can be induced selectively within the nucleus accumbens and dorsal striatum of adult animals (Kelz et al. 1999). Importantly, these mice overexpress ΔFosB selectively in the dynorphin-containing medium spiny neurons, where the drugs are believed to induce the protein. The behavioural phenotype of the ΔFosB-overexpressing mice, which in certain ways resembles animals after chronic drug exposure, is summarized in table 2. The mice show augmented locomotor responses to cocaine after acute and chronic administration (Kelz et al. 1999). They also show enhanced sensitivity to the rewarding effects of cocaine and morphine in place-conditioning assays.
biochemically modified isoforms of by acute administration of a drug of abuse. Also induced are induced in nucleus accumbens and dorsal striatal neurons FosB, (chronic stimulation (1 day after repeated cocaine exposure). Ilation (1–2 hours after a single cocaine exposure) versus family proteins in the nucleus accumbens by acute stimu-

repeated (e.g. twice daily) drug administration, each acute persist in brain for long periods due to their stability. (ii) With are induced at low levels by acute drug administration, but do not overexpress do in nucleus accumbens and dorsal striatum neurons of the nucleus accumbens and dorsal striatum in different lines of bitransgenic mice which fail to show most of these behavioural phenotypes, specifically implicates dynorphin+ nucleus accumbens neurons in these phenomena. In contrast to the overexpression of ΔFosB, overexpression of a mutant Jun protein (ΔJun or ΔJunD)—which functions as a dominant negative antagonist of AP-1-mediated transcription—by the use of bitransgenic mice or viral-mediated gene transfer produces the opposite behavioural effects (Peakman et al. 2003; Zachariou et al. 2006). These data indicate that the induction of ΔFosB in dynorphin-containing medium spiny neurons of the nucleus accumbens increases an animal’s sensitivity to cocaine and other drugs of abuse, and may represent a mechanism for relatively prolonged sensitization to the drugs.

The effects of ΔFosB may extend well beyond the regulation of drug sensitivity per se to more complex behaviours related to the addiction process. Mice overexpressing ΔFosB work harder to self-administer cocaine in progressive ratio self-administration assays, suggesting that ΔFosB may sensitize animals to the incentive motivational properties of cocaine and thereby lead to a propensity for relapse after drug withdrawal (Colby et al. 2003). ΔFosB-overexpressing mice also show enhanced anxiolytic effects of alcohol (Picetti et al. 2001), a phenotype that has been associated with increased alcohol intake in humans. Together, these early findings suggest that ΔFosB, in addition to increasing sensitivity to drugs of abuse, produces qualitative changes in behaviour that promote drug-seeking behaviour, and support the view, stated above, that ΔFosB functions as a sustained molecular switch for the addicted state. An important question under current investigation is whether ΔFosB

Table 1. Drugs of abuse known to induce ΔFosB in nucleus accumbens after chronic administration.

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<tr>
<th>Drug</th>
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<tr>
<td>o呪ates*</td>
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<tr>
<td>cocaine*</td>
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<tr>
<td>amphetamine</td>
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<tr>
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<td>nicotine*</td>
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<td>ethanol*</td>
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<tr>
<td>phencyclidine</td>
</tr>
<tr>
<td>cannabinoids</td>
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*Induction reported for self-administered drug in addition to investigator-administered drug. Drug induction of ΔFosB has been demonstrated in both rats and mice, except the following: mouse only, cannabinoids; rat only, methamphetamine, phencyclidine.

(Felz et al. 1999; Zachariou et al. 2006), and self-administer lower doses of cocaine than littermates that do not overexpress ΔFosB (Colby et al. 2003). As well, ΔFosB overexpression in nucleus accumbens exaggerates the development of opiate physical dependence and promotes opiate analgesic tolerance (Zachariou et al. 2006). By contrast, ΔFosB-expressing mice are normal in several other behavioural domains, including spatial learning as assessed in the Morris water maze (Kelz et al. 1999). Specific targeting of ΔFosB overexpression to the nucleus accumbens, by use of viral-mediated gene transfer, has yielded equivalent data (Zachariou et al. 2006), which indicates that this particular brain region can account for the phenotype observed in the bitransgenic mice, where ΔFosB is also expressed in dorsal striatum and to a lesser extent in certain other brain regions. Moreover, targeting the enkephalin-containing medium spiny neurons in nucleus accumbens and dorsal striatum in different lines of bitransgenic mice which fail to show most of these behavioural phenotypes, specifically implicates dynorphin+ nucleus accumbens neurons in these phenomena. In contrast to the overexpression of ΔFosB, overexpression of a mutant Jun protein (ΔJun or ΔJunD)—which functions as a dominant negative antagonist of AP-1-mediated transcription—by the use of bitransgenic mice or viral-mediated gene transfer produces the opposite behavioural effects (Peakman et al. 2003; Zachariou et al. 2006). These data indicate that the induction of ΔFosB in dynorphin-containing medium spiny neurons of the nucleus accumbens increases an animal’s sensitivity to cocaine and other drugs of abuse, and may represent a mechanism for relatively prolonged sensitization to the drugs.

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accumulation during drug exposure promotes drug-seeking behaviour after extended withdrawal periods, even after ΔFosB levels have normalized (see below).

4. INDUCTION OF ΔFosB IN NUCLEUS ACCUMBENS BY NATURAL REWARDS
The nucleus accumbens is believed to function normally by regulating responses to natural rewards, such as food, drink, sex and social interactions. As a result, there is considerable interest in a possible role of this brain region in so-called natural addictions (e.g. pathological overeating, gambling, exercise, etc.). Animal models of such conditions are limited; nevertheless, we and others have found that high levels of consumption of several types of natural rewards leads to the accumulation of the stable 35–37 kD isoforms of ΔFosB in nucleus accumbens. This has been seen after high levels of wheel running (Werme et al. 2002) as well as after chronic consumption of sucrose, high-fat food or sex (Teegarden & Bale 2007; Wallace et al. 2007; Teegarden et al. in press). In some cases, this induction is selective for the dynorphin+ subset of medium spiny neurons (Werme et al. 2002). Studies of inducible, bitransgenic mice and of viral-mediated gene transfer have demonstrated that overexpression of ΔFosB in nucleus accumbens increases the drive and consumption for these natural rewards, while the overexpression of a dominant negative Jun protein exerts the opposite effect (table 2; Werme et al. 2002; Olsson et al. 2006; Wallace et al. 2007). These findings suggest that ΔFosB in this brain region sensitizes animals not only for drug rewards but for natural rewards as well, and may contribute to states of natural addiction.

5. INDUCTION OF ΔFosB IN NUCLEUS ACCUMBENS BY CHRONIC STRESS
Given the substantial evidence that ΔFosB is induced in nucleus accumbens by chronic exposure to drug and natural rewards, it was interesting to observe that ΔFosB is also highly induced in this brain region after several forms of chronic stress, including restraint stress, chronic unpredictable stress and social defeat (Perrotti et al. 2004; Vialou et al. 2007). Unlike drugs and natural rewards, however, this induction is seen more broadly in this brain region in that it is observed prominently in both dynorphin+ and enkephalin+ subsets of medium spiny neurons. Early evidence suggests that this induction of ΔFosB may represent a positive, coping response that helps an individual adapt to the stress. This hypothesis is supported by preliminary findings that overexpression of ΔFosB in nucleus accumbens, by the use of inducible, bitransgenic mice or viral-mediated gene transfer, exerts antidepressant-like responses in several behavioural assays (e.g. social defeat, forced swim test), while ΔJun expression causes pro-depression-like effects (Vialou et al. 2007). Moreover, the chronic administration of standard antidepressant medications exerts an effect similar to stress and induces ΔFosB in this brain region. While further work is needed to validate these findings, such a role would be consistent with the observations that ΔFosB increases the sensitivity of the brain’s reward circuitry and may thereby help animals cope under periods of stress. Interestingly, this hypothesized role for ΔFosB in nucleus accumbens is similar to that which has been shown recently for periaqueductal grey where the transcription factor is also induced by chronic stress (Berton et al. 2007).

6. TARGET GENES FOR ΔFosB IN NUCLEUS ACCUMBENS
Since ΔFosB is a transcription factor, it presumably produces this interesting behavioural phenotype in nucleus accumbens by enhancing or repressing expression of other genes. As shown in figure 1, ΔFosB is a truncated product of the fosB gene that lacks most of the C-terminal transactivation domain present in full-length FosB but retains the dimerization and DNA-binding domains. ΔFosB binds to Jun family members and the resulting dimer binds AP-1 sites in DNA. Some in vitro studies suggest that because ΔFosB lacks much of its transactivation domain, it functions as a negative regulator of AP-1 activity, while several others show

Table 2. Behavioural phenotype upon ΔFosB induction in dynorphin+ neurons of nucleus accumbens and dorsal striatum.

<table>
<thead>
<tr>
<th>stimulus</th>
<th>phenotype</th>
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<tbody>
<tr>
<td>cocaine</td>
<td>increased locomotor responses to acute administration</td>
</tr>
<tr>
<td>morphine</td>
<td>increased locomotor sensitization to repeated administration</td>
</tr>
<tr>
<td>alcohol</td>
<td>increased incentive motivation in progressive ratio procedure</td>
</tr>
<tr>
<td>wheel running</td>
<td>increased acquisition of cocaine self-administration at lower doses</td>
</tr>
<tr>
<td>sucrose</td>
<td>increased acquisition of cocaine self-administration at lower doses</td>
</tr>
<tr>
<td>high fat</td>
<td>increased acquisition of cocaine self-administration at lower doses</td>
</tr>
<tr>
<td>sex</td>
<td>increased acquisition of cocaine self-administration at lower doses</td>
</tr>
</tbody>
</table>

*The phenotypes described in this table are established upon inducible overexpression of ΔFosB in bitransgenic mice where ΔFosB expression is targeted to dynorphin+ neurons of the nucleus accumbens and dorsal striatum; several-fold lower levels of ΔFosB are seen in hippocampus and frontal cortex. In many cases, the phenotype has been directly linked to ΔFosB expression in nucleus accumbens per se by use of viral-mediated gene transfer.
that ΔFosB can activate transcription at AP-1 sites (Dobrazanski et al. 1991; Nakabepu & Nathans 1991; Yen et al. 1991; Chen et al. 1997).

Using our inducible, bitransgenic mice that overexpress ΔFosB or its dominant negative ΔcJun, and analysing gene expression on Affymetrix chips, we demonstrated that, in the nucleus accumbens in vivo, ΔFosB functions primarily as a transcriptional activator, while it does serve as a repressor for a smaller subset of genes (McClung & Nestler 2003). Interestingly, this differential activity of ΔFosB is a function of the duration and degree of ΔFosB expression, with short-term, lower levels leading to more gene repression and long-term, higher levels leading to more gene activation. This is consistent with the finding that short-term and long-term ΔFosB expressions lead to opposite effects on behaviour: short-term ΔFosB expression, like the expression of ΔcJun, reduces cocaine preference, while longer term ΔFosB expression increases cocaine preference (McClung & Nestler 2003). The mechanism responsible for this shift is currently under investigation; one novel possibility, which remains speculative, is that ΔFosB, at higher levels, may form homodimers that activate AP-1 transcription (Jorissen et al. 2007).

Several target genes of ΔFosB have been established using a candidate gene approach (table 3). One candidate gene is GluR2, an alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor subunit (Kelz et al. 1999). ΔFosB overexpression in inducible bitransgenic mice selectively increases GluR2 expression in nucleus accumbens, with no effect seen on several other AMPA glutamate receptor subunits analysed, while ΔcJun expression blocks the ability of cocaine to upregulate GluR2 (Peakman et al. 2003). AP-1 complexes comprising ΔFosB (and most likely JunD) bind a consensus AP-1 site present in the GluR2 promoter. Furthermore, GluR2 overexpression via viral-mediated gene transfer increases the rewarding effects of cocaine, much like prolonged ΔFosB overexpression (Kelz et al. 1999). Since GluR2-containing AMPA channels have a lower overall conductance compared with AMPA channels that do not contain this subunit, the cocaine- and ΔFosB-mediated upregulation of GluR2 in nucleus accumbens could account, at least in part, for the reduced glutamatergic responses seen in these neurons after chronic drug exposure (Kauer & Malenka 2007; table 3).

Another candidate target gene of ΔFosB in nucleus accumbens is the opioid peptide, dynorphin. Recall that ΔFosB appears to be induced by drugs of abuse specifically in dynorphin-producing cells in this brain region. Drugs of abuse have complex effects on dynorphin expression, with increases or decreases seen depending on the treatment conditions used. The dynorphin gene contains AP-1-like sites, which can bind ΔFosB-containing AP-1 complexes. Moreover, we have shown that the induction of ΔFosB represses dynorphin gene expression in nucleus accumbens (Zachariou et al. 2006). Dynorphin is thought to activate k-opioid receptors on VTA dopamine neurons and inhibit dopaminergic transmission and thereby downregulate reward mechanisms (Shippenberg & Rea 1997). Hence, the ΔFosB repression of dynorphin expression could contribute to the enhancement of reward mechanisms mediated by this transcription factor. There is now direct evidence supporting the involvement of dynorphin gene repression in ΔFosB’s behavioural phenotype (Zachariou et al. 2006).

Recent evidence has shown that ΔFosB also represses the c-fos gene that helps create the molecular switch—from the induction of several short-lived Fos family proteins after acute drug exposure to the predominant accumulation of ΔFosB after chronic drug exposure—cited earlier (Renthal et al. in press). The mechanism responsible for ΔFosB repression of c-fos expression is complex and is covered below.

Table 3. Examples of validated targets for ΔFosB in nucleus accumbensa.

<table>
<thead>
<tr>
<th>target</th>
<th>brain region</th>
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</thead>
<tbody>
<tr>
<td>↑GluR2</td>
<td>decreased sensitivity to glutamate</td>
</tr>
<tr>
<td>↓dynorphinb</td>
<td>downregulation of k-opioid feedback loop</td>
</tr>
<tr>
<td>↑Cdk5</td>
<td>expansion of dendritic processes</td>
</tr>
<tr>
<td>↑NFκB</td>
<td>expansion of dendritic processes; regulation of cell survival pathways</td>
</tr>
<tr>
<td>↓c-Fos</td>
<td>molecular switch from short-lived Fos family proteins induced acutely to ΔFosB induced chronically</td>
</tr>
</tbody>
</table>

*aAlthough ΔFosB regulates the expression of numerous genes in brain (e.g. McClung & Nestler 2003), the table lists only those genes that meet at least three of the following criteria: (i) increased (↑) or decreased (↓) expression upon ΔFosB overexpression, (ii) reciprocal or equivalent regulation by ΔcJun, a dominant negative inhibitor of AP-1-mediated transcription, (iii) ΔFosB-containing AP-1 complexes bind to AP-1 sites in the promoter region of the gene, and (iv) ΔFosB causes a similar effect on gene promoter activity in vitro as seen in vivo.

bDespite evidence that ΔFosB represses the dynorphin gene in drug abuse models (Zachariou et al. 2006), there is other evidence that it may act to activate the gene under different circumstances (see Cenci 2002).
nucleus accumbens

core + + + +
shell + + + +
dorsal striatum + + + +
ventral pallidum\(^b\) n.d. + + n.d. +
prefrontal cortex\(^c\) + + + +
lateral septum + + + +
medial septum + + + +
BNST + + + +
IPAC + + + +

hippocampus
dentate gyrus + + + +
CA1 + + + +
CA3 + + + +
amygdala
basolateral + + + +
central + + + +
medial + + + +
periaqueductal grey + + + +
ventral tegmental area + + + +
substantia nigra + + + +

<table>
<thead>
<tr>
<th>REGIONS</th>
<th>cocaine</th>
<th>morphine</th>
<th>ethanol</th>
<th>cannabinoids</th>
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<tbody>
<tr>
<td>nucleus accumbens</td>
<td>core</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>shell</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>dorsal striatum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>ventral pallidum(^b)</td>
<td>n.d.</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
</tr>
<tr>
<td>prefrontal cortex(^c)</td>
<td>+</td>
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<tr>
<td>lateral septum</td>
<td>+</td>
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<tr>
<td>medial septum</td>
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<td>BNST</td>
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<td>IPAC</td>
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\(^a\)The table does not show the relative levels of ΔFosB induction by the various drugs. See Perrotti et al. (2008) for this information.

\(^b\)The effect of cocaine, ethanol and cannabinoids on ΔFosB induction in ventral pallidium has not yet been studied, but such induction has been observed in response to methamphetamine (McDaid et al. 2006b).

\(^c\)ΔFosB induction is seen in several subregions of prefrontal cortex, including infralimbic (medial prefrontal) and orbitofrontal cortex.

Another ΔFosB target identified by use of microarrays is NFκB. This transcription factor is induced in nucleus accumbens by ΔFosB overexpression and chronic cocaine, an effect blocked by ΔJun expression (Ang et al. 2001; Peakman et al. 2003). Recent evidence has suggested that the induction of NFκB may also contribute to cocaine’s ability to induce dendritic spines in nucleus accumbens neurons (Russo et al. 2007). In addition, NFκB has been implicated in some of the neurotoxic effects of methamphetamine in striatal regions (Asanuma & Cadet 1998). The observation that NFκB is a target gene for ΔFosB emphasizes the complexity of the mechanisms by which ΔFosB mediates the effects of cocaine on gene expression. Thus, in addition to the genes regulated by ΔFosB directly via AP-1 sites on the gene promoters, ΔFosB would be expected to regulate many additional genes via altered expression of NFκB and presumably other transcriptional regulatory proteins.

The DNA expression arrays provide a rich list of many additional genes that may be targeted, directly or indirectly, by ΔFosB. Among these genes are additional neurotransmitter receptors, proteins involved in presynaptic and postsynaptic functions, many types of ion channels and intracellular signalling proteins, as well as proteins that regulate the neuronal cytoskeleton and cell growth (McClung & Nestler 2003). Further work is needed to confirm each of these numerous proteins as bona fide targets of cocaine acting through ΔFosB and to establish the precise role that each protein plays in mediating the complex neural and behavioural aspects of cocaine action. Ultimately, of course, it will be crucial to move beyond the analysis of individual target genes to the regulation of groups of genes whose coordinated regulation is probably required to mediate the addicted state.

7. INDUCTION OF ΔFosB IN OTHER BRAIN REGIONS

The discussion up to now has focused solely on nucleus accumbens. While this is a key brain reward region and important for the addicting actions of cocaine and other drugs of abuse, many other brain regions are also crucial in the development and maintenance of a state of addiction. An important question, then, is whether ΔFosB acting in other brain regions beyond the nucleus accumbens may also influence drug addiction. Indeed, there is now increasing evidence that stimulant and opiate drugs of abuse induce ΔFosB in several brain regions implicated in diverse aspects of addiction (Nye et al. 1995; Perrotti et al. 2005, 2008; McDaid et al. 2006a,b; Liu et al. 2007).

A recent study has systematically compared ΔFosB induction in these various brain regions across four different drugs of abuse: cocaine; morphine; cannabinoids; and ethanol (table 4; Perrotti et al. 2008). All four drugs induce the transcription factor to varying degrees in nucleus accumbens and dorsal striatum as well as in prefrontal cortex, amygdala, hippocampus, bed nucleus of the stria terminalis and interstitial nucleus of the posterior limb of the anterior commissure.
Cocaine and ethanol alone induce ΔFosB in lateral septum, all of the drugs except for cannabinoids induce ΔFosB in the peri-aqueductual grey, and cocaine is unique in inducing ΔFosB in gamma-amino butyric acid (GABA)ergic cells in the posterior ventral tegmental area (Perrotti et al. 2005, 2008). In addition, morphine has been shown to induce ΔFosB in ventral pallidum (McDaid et al. 2006a). In each of these regions, it is the 35–37 kD isoforms of ΔFosB that accumulate with chronic drug exposure and persist for relatively long periods during withdrawal.

A major goal for future research is to carry out studies, analogous to those described above for nucleus accumbens, to delineate the neural and behavioural phenotypes mediated by ΔFosB for each of these brain regions. This represents an enormous undertaking, yet it is crucial for understanding the global influence of ΔFosB on the addiction process.

We have recently taken a significant step in this regard by using viral-mediated gene transfer to characterize the actions of ΔFosB in a subregion of prefrontal cortex, namely, orbitofrontal cortex. This region has been strongly implicated in addiction, in particular, in contributing to the impulsivity and compulsivity that characterize an addicted state (Kalivas & Volkow 2005). Interestingly, unlike the nucleus accumbens where self-administered and yoked cocaine induce comparable levels of ΔFosB as noted earlier, we observed that cocaine self-administration causes a several-fold greater induction of ΔFosB in orbitofrontal cortex, suggesting that this response may be related to volitional aspects of drug administration (Winstanley et al. 2007). We then used rodent tests of attention and decision-making (e.g. five-choice serial reaction time and delay-discounting tests) to determine whether ΔFosB within the orbitofrontal cortex contributes to drug-induced alterations in cognition. We found that chronic cocaine treatment produces tolerance to the cognitive impairments caused by acute cocaine. Viral-mediated overexpression of ΔFosB within this region mimicked the effects of chronic cocaine, while overexpression of the dominant negative antagonist, ΔJunD, prevents this behavioural change. DNA expression microarray analyses identified several potential molecular mechanisms underlying this behavioural change, including a cocaine- and ΔFosB-mediated increase in transcription of the metabotropic glutamate receptor mGlur5 and GABA A receptor as well as substance P (Winstanley et al. 2007). The influence of these and many other putative ΔFosB targets requires further investigation.

These findings indicate that ΔFosB helps mediate tolerance to the cognitive-disrupting effects of cocaine. Users who experience tolerance to the deleterious effects of cocaine are more likely to become cocaine dependent, whereas those who find the drug more disruptive at work or school are less likely to become addicted (Shaffer & Eber 2002). Tolerance to the cognitive disruption caused by acute cocaine in cocaine-experienced individuals may therefore facilitate the maintenance of addiction. In this way, ΔFosB induction in the orbitofrontal cortex may promote an addicted state, similar to its actions in the nucleus accumbens where ΔFosB promotes addiction by enhancing the rewarding and incentive motivational effects of the drug.

8. EPIGENETIC MECHANISMS OF ΔFosB ACTION

Until recently, all studies of transcriptional regulation in brain have relied on measurements of steady-state mRNA levels. For example, the search for ΔFosB target genes has involved identifying mRNA’s up- or down-regulated upon ΔFosB or ΔJun overexpression, as stated earlier. This level of analysis has been very useful in identifying putative targets for ΔFosB; however, it is inherently limited in providing insight into the underlying mechanisms involved. Rather, all studies of mechanisms have relied on in vitro measures such as ΔFosB binding to a gene’s promoter sequences in gel shift assays or ΔFosB regulation of a gene’s promoter activity in cell culture. This is unsatisfying because mechanisms of transcription regulation show dramatic variations from cell type to cell type, leaving it virtually completely unknown how a drug of abuse, or ΔFosB, regulates its specific genes in the brain in vivo.

Studies of epigenetic mechanisms make it possible, for the first time, to push the envelope one step further and directly examine transcriptional regulation in the brains of behaving animals (Tsankova et al. 2007). Historically, the term epigenetics describes mechanisms by which cellular traits can be inherited without a change in DNA sequence. We use the term more broadly to encompass ‘the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states’ (Bird 2007). Thus, we now know that the activity of genes is controlled by the covalent modification (e.g. acetylation, methylation) of histones in the genes’ vicinity and the recruitment of diverse types of coactivators or corepressors of transcription. Chromatin immunoprecipitation (ChIP) assays make it possible to take advantage of this growing knowledge of chromatin biology to determine the activation state of a gene in a particular brain region of an animal treated with a drug of abuse.

Examples of how studies of chromatin regulation can help us understand the detailed molecular mechanisms of the action of cocaine and ΔFosB are given in figure 3. As stated above, ΔFosB can function as either a transcriptional activator or repressor depending on the target gene involved. To gain insight into these actions, we analysed the chromatin state of two representative gene targets for ΔFosB, cdk5 that is induced by ΔFosB and c-fos that is repressed in nucleus accumbens. Chromatin immunoprecipitation studies demonstrated that cocaine activates the cdk5 gene in this brain region through the following cascade: ΔFosB binds to the cdk5 gene and then recruits histone acetyltransferases (HAT; which acetylate nearby histones) and SWI–SNF factors; both actions promote gene transcription (Kumar et al. 2005; Levine et al. 2005). Chronic cocaine further augments histone acetylation through the phosphorylation and inhibition of histone deacetylases (HDAC; which normally deacetylase and repress genes; Renthal et al. 2007). By contrast, cocaine represses the c-fos gene; when ΔFosB binds to this gene it recruits an HDAC and possibly histone methyltransferases (HMT; which

Phil. Trans. R. Soc. B (2008)
methylation, and methylation, respectively. A, P, and M depict histone acetylation, HDAC as well as perhaps HMTs which repress gene expression. A, P, and M depict histone acetylation, HDAC, and SWI–SNF factors, which promote gene activation. There is also evidence for exclusion of HDACs (see text). (a) At the cdk5 promoter, ∆FosB recruits HAT and SWI–SNF factors, which promote gene activation. (b) By contrast, at the c-fos promoter, ∆FosB recruits HDAC1 as well as perhaps HMTs which repress gene expression. A, P, and M depict histone acetylation, phosphorylation, and methylation, respectively.

Figure 3. Epigenetic mechanisms of ∆FosB action. The figure illustrates the very different consequences when ∆FosB binds to a gene that it activates (e.g. cdk5) versus represses (e.g. c-fos). (a) At the cdk5 promoter, ∆FosB recruits HAT and SWI–SNF factors, which promote gene activation. There is also evidence for exclusion of HDACs (see text). (b) By contrast, at the c-fos promoter, ∆FosB recruits HDAC1 as well as perhaps HMTs which repress gene expression. A, P, and M depict histone acetylation, phosphorylation, and methylation, respectively.

9. CONCLUSIONS

The pattern of induction of ∆FosB in nucleus accumbens by chronic exposure to natural rewards, stress or drugs of abuse raises an interesting hypothesis concerning the protein’s normal functioning in this brain region. As depicted in figure 2, there is an appreciable level of ∆FosB in nucleus accumbens under normal conditions. This is unique to striatal regions, and ∆FosB is virtually undetectable elsewhere throughout brain at baseline. We hypothesize that levels of ∆FosB in nucleus accumbens represent a read-out of an individual’s exposure to emotional stimuli, both positive and negative, integrated over relatively long periods of time given the temporal properties of the protein. The partial differences in the cellular specificity of ∆FosB induction by rewarding versus aversive stimuli are poorly understood, and further work is needed to elucidate the functional consequences of these distinctions. We hypothesize further that as higher levels of emotional stimulation induce more ∆FosB in nucleus accumbens neurons, the neurons’ functioning is altered so that they become more sensitive to rewarding stimuli. In this way, induction of ∆FosB would promote reward-related (i.e. emotional) memory through afferent projects of the nucleus accumbens. Under normal circumstances, the induction of moderate levels of ∆FosB by rewarding or aversive stimuli would be adaptive by enhancing an animal’s adjustments to environmental challenges. However, the excessive induction of ∆FosB seen under pathological conditions (e.g. chronic exposure to a drug of abuse) would lead to excessive sensitization of the nucleus accumbens circuitry and ultimately contribute to pathological behaviours (e.g. compulsive drug seeking and taking) associated with drug addiction. ∆FosB induction in other brain regions would presumably contribute to distinct aspects of an addicted state, as have been suggested by recent findings of ∆FosB action in orbitofrontal cortex.

If this hypothesis is correct, it raises the interesting possibility that levels of ∆FosB in nucleus accumbens or perhaps other brain regions could be used as a biomarker to assess the state of activation of an individual’s reward circuitry, as well as the degree to which an individual is ‘addicted’, both during the development of an addiction and its gradual waning during extended withdrawal or treatment. The use of ∆FosB as a marker of a state of addiction has been demonstrated in animal models. Adolescent animals show much greater induction of ∆FosB compared with older animals, consistent with their greater vulnerability for addiction (Ehrlich et al. 2002). In addition, attenuation of the rewarding effects of nicotine with a GABA<sub>B</sub> receptor positive allosteric modulator is associated with the blockade of nicotine induction of ∆FosB in nucleus accumbens (Momberbeau et al. 2007). Although highly speculative, it is conceivable that a small molecule PET ligand, with high affinity for ∆FosB, could be used to help diagnose addictive disorders as well as monitor progress during treatment.

Finally, ∆FosB itself or any of the numerous genes it regulates—identified through DNA expression arrays or ChIP on chip assays—represent potential targets for the development of fundamentally novel treatments for drug addiction. We believe that it is imperative to look beyond traditional drug targets (e.g. neurotransmitter receptors and transporters) for...
potential treatment agents for addiction. The genome-wide transcriptional maps capable of today's advanced technologies provide a promising source of such novel targets in our efforts to better treat and ultimately cure addictive disorders.

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REFERENCES


