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Review

Transcriptional mechanisms of addiction: role of ΔFosB

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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

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Figure 1. Biochemical basis of ΔFosB's unique stability: (*a*) FosB (338 aa, M_r approx. 38 kD) and (*b*) ΔFosB (237 aa, M_r approx. 26 kD) are encoded by the *fosB* gene. ΔFosB is generated by alternative splicing and lacks the C-terminal 101 amino acids present in FosB. Two mechanisms are known that account for ΔFosB's stability. First, ΔFosB lacks two degron domains present in the C-terminus of full-length FosB (and found in all other Fos family proteins as well). One of these degron domains targets FosB for ubiquitination and degradation in the proteasome. The other degron domain targets FosB degradation by a ubiquitin- and proteasome-independent mechanism. Second, ΔFosB is phosphorylated by casein kinase 2 (CK2) and probably by other protein kinases (?) at its N-terminus, which further stabilizes the protein.

responses are seen 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_r 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; McDaid *et al.* 2006*b*), 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

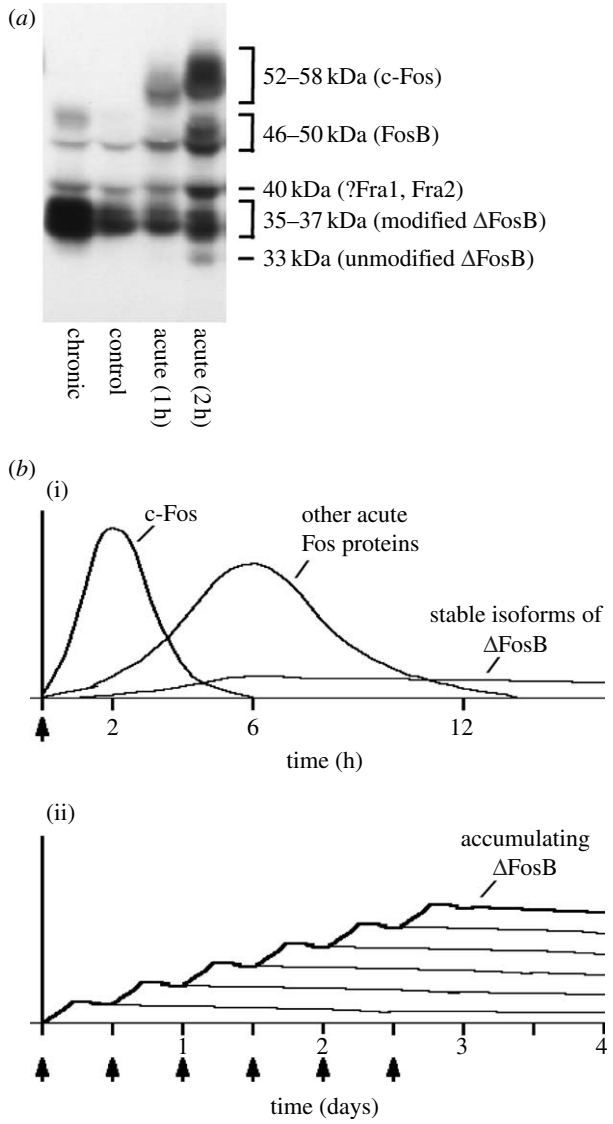

Figure 2. Scheme showing the gradual accumulation of ΔFosB versus the rapid and transient induction of other Fos family proteins in response to drugs of abuse. (*a*) The autoradiogram illustrates the differential induction of Fos family proteins in the nucleus accumbens by acute stimulation (1–2 hours after a single cocaine exposure) versus chronic stimulation (1 day after repeated cocaine exposure). (*b*) (i) Several waves of Fos family proteins (comprising c-Fos, FosB, ΔFosB (33 kD isoform), and possibly (?) Fra1, Fra2) are induced in nucleus accumbens and dorsal striatal neurons by acute administration of a drug of abuse. Also induced are biochemically modified isoforms of ΔFosB (35–37 kD); they are induced at low levels by acute drug administration, but persist in brain for long periods due to their stability. (ii) With repeated (e.g. twice daily) drug administration, each acute stimulus induces a low level of the stable ΔFosB isoforms. This is indicated by the lower set of overlapping lines that indicate ΔFosB induced by each acute stimulus. The result is a gradual increase in the total levels of ΔFosB with repeated stimuli during a course of chronic treatment. This is indicated by the increasing stepped line in the graph.

(Kelz *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).

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

opiates[a]
cocaine[a]
amphetamine
methamphetamine
nicotine[a]
ethanol[a]
phencyclidine
cannabinoids

[a] 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.

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 (ΔcJun 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 2. Behavioural phenotype upon ΔFosB induction in dynorphin+ neurons of nucleus accumbens and dorsal striatum[a].

stimulus	phenotype
cocaine	increased locomotor responses to acute administration
	increased locomotor sensitization to repeated administration
	increased conditioned place preference at lower doses
	increased acquisition of cocaine self-administration at lower doses
	increased incentive motivation in progressive ratio procedure
morphine	increased conditioned place preference at lower drug doses
	increased development of physical dependence and withdrawal
	decreased initial analgesic responses, enhanced tolerance
alcohol	increased anxiolytic responses
wheel running	increased wheel running
sucrose	increased incentive for sucrose in progressive ratio procedure
high fat	increased anxiety-like responses upon withdrawal of high-fat diet
sex	increased sexual behaviour

[a] 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.

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; Olausson *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 ΔcJun 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 3. Examples of validated targets for ΔFosB in nucleus accumbens[a].

target	brain region
↑GluR2	decreased sensitivity to glutamate
↓dynorphin[b]	downregulation of κ-opioid feedback loop
↑Cdk5	expansion of dendritic processes
↑NFκB	expansion of dendritic processes; regulation of cell survival pathways
↓c-Fos	molecular switch from short-lived Fos family proteins induced acutely to ΔFosB induced chronically

[a]Although 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*.
[b]Despite 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).

that ΔFosB can activate transcription at AP-1 sites (Dobrazanski *et al*. 1991; Nakabeppu & 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 κ-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.

Another approach used to identify target genes of ΔFosB has measured the gene expression changes that occur upon the inducible overexpression of ΔFosB (or ΔcJun) in nucleus accumbens using DNA expression arrays, as described earlier. This approach has led to the identification of many genes that are up- or downregulated by ΔFosB expression in this brain region (Chen *et al*. 2000, 2003; Ang *et al*. 2001; McClung & Nestler 2003). Two genes that appear to be induced through ΔFosB's actions as a transcriptional activator are cyclin-dependent kinase-5 (Cdk5) and its cofactor P35 (Bibb *et al*. 2001; McClung & Nestler 2003). Cdk5 is also induced by chronic cocaine in the nucleus accumbens, an effect blocked upon ΔcJun expression, and ΔFosB binds to and activates the Cdk5 gene through an AP-1 site in its promoter (Chen *et al*. 2000; Peakman *et al*. 2003). Cdk5 is an important target of ΔFosB since its expression has been directly linked to changes in the phosphorylation state of

Table 4. Comparison of brain regions that show ΔFosB induction after chronic exposure to representative drugs of abuse[a].

	cocaine	morphine	ethanol	cannabinoids
nucleus accumbens				
core	+	+	+	+
shell	+	+	+	+
dorsal striatum	+	+	+	+
ventral pallidum[b]	n.d.	+	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	−	−	−	−

[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 pallidum has not yet been studied, but such induction has been observed in response to methamphetamine (McDaid *et al.* 2006*b*).
[c]ΔFosB induction is seen in several subregions of prefrontal cortex, including infralimbic (medial prefrontal) and orbitofrontal cortex.

numerous synaptic proteins including glutamate receptor subunits (Bibb *et al.* 2001), as well as increases in dendritic spine density (Norrholm *et al.* 2003; Lee *et al.* 2006), in the nucleus accumbens, which are associated with chronic cocaine administration (Robinson & Kolb 2004). Recently, the regulation of Cdk5 activity in nucleus accumbens has been directly linked to alterations in the behavioural effects of cocaine (Taylor *et al.* 2007).

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 ΔcJun 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 pre- 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.* 2006*a*,*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 periaqueductal grey, and cocaine is unique in inducing ΔFosB in gamma-aminobutyric 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.* 2006*a*). 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 adaptation. DNA expression microarray analyses identified several potential molecular mechanisms underlying this behavioural change, including a cocaine- and ΔFosB-mediated increase in transcription of the metabotrophic 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 ΔcJun 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 deacetylate 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

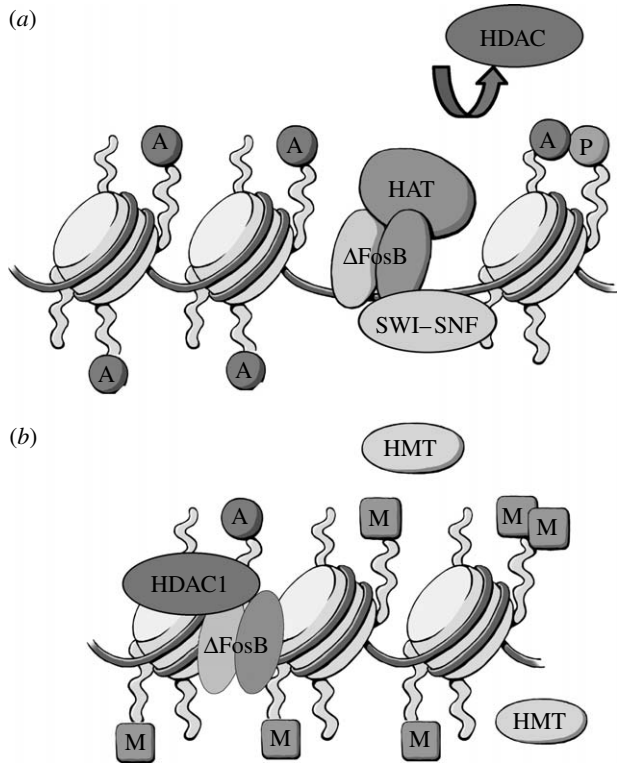

Figure 3. Epigenetic mechanisms of ΔFosB action. The figure illustrates the very different consequences when ΔFosB binds to a gene that it activates (e.g. *cdk5*) versus represses (e.g. *c-fos*). (*a*) At the *cdk5* promoter, ΔFosB recruits HAT and SWI–SNF factors, which promote gene activation. There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

methylate nearby histones) and thereby inhibits *c-fos* transcription (figure 3; Renthal *et al.* in press). A central question is: what determines whether ΔFosB activates or represses a gene when it binds to that gene's promoter?

These early studies of epigenetic mechanisms of drug addiction are exciting because they promise to reveal fundamentally new information concerning the molecular mechanisms by which drugs of abuse regulate gene expression in nucleus accumbens and other brain regions. Combining DNA expression arrays with so-called ChIP on chip assays (where alterations in chromatin structure or transcription factor binding can be analysed genome wide) will lead to the identification of drug and ΔFosB target genes with much greater levels of confidence and completeness. In addition, epigenetic mechanisms are particularly attractive candidates to mediate the very long-lived phenomena central to a state of addiction. In this way, drug- and ΔFosB-induced changes in histone modifications and related epigenetic alterations provide potential mechanisms by which transcriptional changes can persist long after drug exposure ceases and perhaps even after ΔFosB degrades to normal levels.

9. CONCLUSIONS

The pattern of induction of ΔFosB 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 ΔFosB in nucleus accumbens under normal conditions. This is unique to striatal regions, as ΔFosB is virtually undetectable elsewhere throughout brain at baseline. We hypothesize that levels of ΔFosB 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 ΔFosB 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 ΔFosB in nucleus accumbens neurons, the neurons' functioning is altered so that they become more sensitive to rewarding stimuli. In this way, induction of ΔFosB would promote reward-related (i.e. emotional) memory through afferent projects of the nucleus accumbens. Under normal circumstances, the induction of moderate levels of ΔFosB by rewarding or aversive stimuli would be adaptive by enhancing an animal's adjustments to environmental challenges. However, the excessive induction of ΔFosB 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. ΔFosB induction in other brain regions would presumably contribute to distinct aspects of an addicted state, as have been suggested by recent findings of ΔFosB action in orbitofrontal cortex.

If this hypothesis is correct, it raises the interesting possibility that levels of ΔFosB 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 ΔFosB as a marker of a state of addiction has been demonstrated in animal models. Adolescent animals show much greater induction of ΔFosB 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 $GABA_B$ receptor positive allosteric modulator is associated with the blockade of nicotine induction of ΔFosB in nucleus accumbens (Mombereau *et al.* 2007). Although highly speculative, it is conceivable that a small molecule PET ligand, with high affinity for ΔFosB, could be used to help diagnose addictive disorders as well as monitor progress during treatment.

Finally, ΔFosB 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

Alibhai, I. N., Green, T. A., Potashkin, J. A. & Nestler, E. J. 2007 Regulation of *fosB* and *ΔfosB* mRNA expression: *in vivo* and *in vitro* studies. *Brain Res.* **1143**, 22–33. (doi:10.1016/j.brainres.2007.01.069)

Ang, E., Chen, J., Zagouras, P., Magna, H., Holland, J., Schaeffer, E. & Nestler, E. J. 2001 Induction of NFκB in nucleus accumbens by chronic cocaine administration. *J. Neurochem.* **79**, 221–224. (doi:10.1046/j.1471-4159.2001.00563.x)

Asanuma, M. & Cadet, J. L. 1998 Methamphetamine-induced increase in striatal NFκB DNA-binding activity is attenuated in superoxide dismutase transgenic mice. *Mol. Brain Res.* **60**, 305–309. (doi:10.1016/S0169-328X(98)00188-0)

Berton, O. *et al*. 2007 Induction of ΔFosB in the periaqueductal gray by stress promotes active coping responses. *Neuron* **55**, 289–300. (doi:10.1016/j.neuron.2007.06.033)

Bibb, J. A. *et al*. 2001 Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* **410**, 376–380. (doi:10.1038/35066591)

Bird, A. 2007 Perceptions of epigenetics. *Nature* **447**, 396–398. (doi:10.1038/nature05913)

Carle, T. L., Ohnishi, Y. N., Ohnishi, Y. H., Alibhai, I. N., Wilkinson, M. B., Kumar, A. & Nestler, E. J. 2007 Absence of conserved C-terminal degron domain contributes to ΔFosB's unique stability. *Eur. J. Neurosci.* **25**, 3009–3019. (doi:10.1111/j.1460-9568.2007.05575.x)

Carlezon Jr, W. A., Duman, R. S. & Nestler, E. J. 2005 The many faces of CREB. *Trends Neurosci.* **28**, 436–445. (doi:10.1016/j.tins.2005.06.005)

Cenci, M. A. 2002 Transcription factors involved in the pathogenesis of L-DOPA-induced dyskinesia in a rat model of Parkinson's disease. *Amino Acids* **23**, 105–109.

Chen, J. S., Nye, H. E., Kelz, M. B., Hiroi, N., Nakabeppu, Y., Hope, B. T. & Nestler, E. J. 1995 Regulation of ΔFosB and FosB-like proteins by electroconvulsive seizure (ECS) and cocaine treatments. *Mol. Pharmacol.* **48**, 880–889.

Chen, J., Kelz, M. B., Hope, B. T., Nakabeppu, Y. & Nestler, E. J. 1997 Chronic FRAs: stable variants of ΔFosB induced in brain by chronic treatments. *J. Neurosci.* **17**, 4933–4941.

Chen, J. S., Zhang, Y. J., Kelz, M. B., Steffen, C., Ang, E. S., Zeng, L. & Nestler, E. J. 2000 Induction of cyclin-dependent kinase 5 in hippocampus by chronic electroconvulsive seizures: role of ΔFosB. *J. Neurosci.* **20**, 8965–8971.

Chen, J., Newton, S. S., Zeng, L., Adams, D. H., Dow, A. L., Madsen, T. M., Nestler, E. J. & Duman, R. S. 2003 Downregulation of the CCAAT-enhancer binding protein beta in ΔFosB transgenic mice and by electroconvulsive seizures. *Neuropsychopharmacology* **29**, 23–31. (doi:10.1038/sj.npp.1300289)

Colby, C. R., Whisler, K., Steffen, C., Nestler, E. J. & Self, D. W. 2003 ΔFosB enhances incentive for cocaine. *J. Neurosci.* **23**, 2488–2493.

Deroche-Gamonet, V. *et al*. 2003 The glucocorticoid receptor as a potential target to reduce cocaine abuse. *J. Neurosci.* **23**, 4785–4790.

Dobrazanski, P., Noguchi, T., Kovary, K., Rizzo, C. A., Lazo, P. S. & Bravo, R. 1991 Both products of the *fosB* gene, FosB and its short form, FosB/SF, are transcriptional activators in fibroblasts. *Mol. Cell Biol.* **11**, 5470–5478.

Ehrlich, M. E., Sommer, J., Canas, E. & Unterwald, E. M. 2002 Periadolescent mice show enhanced ΔFosB upregulation in response to cocaine and amphetamine. *J. Neurosci.* **22**, 9155–9159.

Graybiel, A. M., Moratalla, R. & Robertson, H. A. 1990 Amphetamine and cocaine induce drug-specific activation of the *c-fos* gene in striosome-matrix compartments and limbic subdivisions of the striatum. *Proc. Natl Acad. Sci. USA* **87**, 6912–6916. (doi:10.1073/pnas.87.17.6912)

Green, T. A., Alibhai, I. N., Hommel, J. D., DiLeone, R. J., Kumar, A., Theobald, D. E., Neve, R. L. & Nestler, E. J. 2006 Induction of ICER expression in nucleus accumbens by stress or amphetamine increases behavioral responses to emotional stimuli. *J. Neurosci.* **26**, 8235–8242.

Green, T. A., Alibhai, I. N., Unterberg, S., Neve, R. L., Ghose, S., Tamminga, C. A. & Nestler, E. J. 2008 Induction of activating transcription factors (ATFs) ATF2, ATF3, and ATF4 in the nucleus accumbens and their regulation of emotional behavior. *J. Neurosci.* **28**, 2025–2032. (doi:10.1523/JNEUROSCI.5273-07.2008)

Hiroi, N., Brown, J., Haile, C., Ye, H., Greenberg, M. E. & Nestler, E. J. 1997 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* **94**, 10 397–10 402. (doi:10.1073/pnas.94.19.10397)

Hiroi, N., Brown, J., Ye, H., Saudou, F., Vaidya, V. A., Duman, R. S., Greenberg, M. E. & Nestler, E. J. 1998 Essential role of the *fosB* gene in molecular, cellular, and behavioral actions of electroconvulsive seizures. *J. Neurosci.* **18**, 6952–6962.

Hope, B., Kosofsky, B., Hyman, S. E. & Nestler, E. J. 1992 Regulation of IEG expression and AP-1 binding by chronic cocaine in the rat nucleus accumbens. *Proc. Natl Acad. Sci. USA* **89**, 5764–5768. (doi:10.1073/pnas.89.13.5764)

Hope, B. T., Nye, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., Duman, R. S. & Nestler, E. J. 1994 Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* **13**, 1235–1244. (doi:10.1016/0896-6273(94)90061-2)

Jorissen, H., Ulery, P., Henry, L., Gourneni, S., Nestler, E. J. & Rudenko, G. 2007 Dimerization and DNA-binding properties of the transcription factor ΔFosB. *Biochemistry* **46**, 8360–8372. (doi:10.1021/bi700494v)

Kalivas, P. W. & Volkow, N. D. 2005 The neural basis of addiction: a pathology of motivation and choice. *Am. J. Psychiatry* **162**, 1403–1413. (doi:10.1176/appi.ajp.162.8.1403)

Kauer, J. A. & Malenka, R. C. 2007 Synaptic plasticity and addiction. *Nat. Rev. Neurosci.* **8**, 844–858. (doi:10.1038/nrn2234)

Kelz, M. B. *et al*. 1999 Expression of the transcription factor ΔFosB in the brain controls sensitivity to cocaine. *Nature* **401**, 272–276. (doi:10.1038/45790)

Kumar, A. *et al*. 2005 Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* **48**, 303–314. (doi:10.1016/j.neuron.2005.09.023)

Lee, K. W., Kim, Y., Kim, A. M., Helmin, K., Nairn, A. C. & Greengard, P. 2006 Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. *Proc. Natl Acad. Sci. USA* **103**, 3399–3404. (doi:10.1073/pnas.0511244103)

Levine, A., Guan, Z., Barco, A., Xu, S., Kandel, E. & Schwartz, J. 2005 CREB-binding protein controls response to cocaine by acetylating histones at the *fosB* promoter in the mouse striatum. *Proc. Natl Acad. Sci. USA* **102**, 19 186–19 191. (doi:10.1073/pnas.0509735102)

Liu, H. F., Zhou, W. H., Zhu, H. Q., Lai, M. J. & Chen, W. S. 2007 Microinjection of M(5) muscarinic receptor antisense oligonucleotide into VTA inhibits FosB expression in the NAc and the hippocampus of heroin sensitized rats. *Neurosci. Bull.* **23**, 1–8. (doi:10.1007/s12264-007-0001-6)

Mackler, S. A., Korutla, L., Cha, X. Y., Koebbe, M. J., Fournier, K. M., Bowers, M. S. & Kalivas, P. W. 2000 NAC-1 is a brain POZ/BTB protein that can prevent cocaine-induced sensitization in the rat. *J. Neurosci.* **20**, 6210–6217.

McClung, C. A. & Nestler, E. J. 2003 Regulation of gene expression and cocaine reward by CREB and ΔFosB. *Nat. Neurosci.* **11**, 1208–1215. (doi:10.1038/nn1143)

McClung, C. A., Ulery, P. G., Perrotti, L. I., Zachariou, V., Berton, O. & Nestler, E. J. 2004 ΔFosB: a molecular switch for long-term adaptation in the brain. *Mol. Brain Res.* **132**, 146–154. (doi:10.1016/j.molbrainres.2004.05.014)

McDaid, J., Dallimore, J. E., Mackie, A. R. & Napier, T. C. 2006*a* Changes in accumbal and pallidal pCREB and ΔFosB in morphine-sensitized rats: correlations with receptor-evoked electrophysiological measures in the ventral pallidum. *Neuropsychopharmacology* **31**, 1212–1226.

McDaid, J., Graham, M. P. & Napier, T. C. 2006*b* Methamphetamine-induced sensitization differentially alters pCREB and ΔFosB throughout the limbic circuit of the mammalian brain. *Mol. Pharmacol.* **70**, 2064–2074. (doi:10.1124/mol.106.023051)

Mombereau, C., Lhuillier, L., Kaupmann, K. & Cryan, J. F. 2007 GABAB receptor-positive modulation-induced blockade of the rewarding properties of nicotine is associated with a reduction in nucleus accumbens ΔFosB accumulation. *J. Pharmacol. Exp. Therapy* **321**, 172–177. (doi:10.1124/jpet.106.116228)

Moratalla, R., Elibol, R., Vallejo, M. & Graybiel, A. M. 1996 Network-level changes in expression of inducible Fos–Jun proteins in the striatum during chronic cocaine treatment and withdrawal. *Neuron* **17**, 147–156. (doi:10.1016/S0896-6273(00)80288-3)

Morgan, J. I. & Curran, T. 1995 Immediate-early genes: ten years on. *Trends Neurosci.* **18**, 66–67. (doi:10.1016/0166-2236(95)93874-W)

Muller, D. L. & Unterwald, E. M. 2005 D1 dopamine receptors modulate ΔFosB induction in rat striatum after intermittent morphine administration. *J. Pharmacol. Exp. Therapy* **314**, 148–155. (doi:10.1124/jpet.105.083410)

Nakabeppu, Y. & Nathans, D. 1991 A naturally occurring truncated form of FosB that inhibits Fos/Jun transcriptional activity. *Cell* **64**, 751–759. (doi:10.1016/0092-8674(91)90504-R)

Nestler, E. J. 2001 Molecular basis of long-term plasticity underlying addiction. *Nat. Rev. Neurosci.* **2**, 119–128. (doi:10.1038/35053570)

Nestler, E. J., Barrot, M. & Self, D. W. 2001 ΔFosB: a sustained molecular switch for addiction. *Proc. Natl Acad. Sci. USA* **98**, 11 042–11 046. (doi:10.1073/pnas.191352698)

Norrholm, S. D., Bibb, J. A., Nestler, E. J., Ouimet, C. C., Taylor, J. R. & Greengard, P. 2003 Cocaine-induced proliferation of dendritic spines in nucleus accumbens is dependent on the activity of cyclin-dependent kinase-5. *Neuroscience* **116**, 19–22. (doi:10.1016/S0306-4522(02)00560-2)

Nye, H. E. & Nestler, E. J. 1996 Induction of chronic Fras (Fos-related antigens) in rat brain by chronic morphine administration. *Mol. Pharmacol.* **49**, 636–645.

Nye, H., Hope, B. T., Kelz, M., Iadarola, M. & Nestler, E. J. 1995 Pharmacological studies of the regulation by cocaine of chronic Fra (Fos-related antigen) induction in the striatum and nucleus accumbens. *J. Pharmacol. Exp. Therapy* **275**, 1671–1680.

O'Donovan, K. J., Tourtellotte, W. G., Millbrandt, J. & Baraban, J. M. 1999 The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience. *Trends Neurosci.* **22**, 167–173. (doi:10.1016/S0166-2236(98)01343-5)

Olausson, P., Jentsch, J. D., Tronson, N., Neve, R., Nestler, E. J. & Taylor, J. R. 2006 ΔFosB in the nucleus accumbens regulates food-reinforced instrumental behavior and motivation. *J. Neurosci.* **26**, 9196–9204. (doi:10.1523/JNEUROSCI.1124-06.2006)

Peakman, M.-C. *et al.* 2003 Inducible, brain region specific expression of a dominant negative mutant of c-Jun in transgenic mice decreases sensitivity to cocaine. *Brain Res.* **970**, 73–86. (doi:10.1016/S0006-8993(03)02230-3)

Pérez-Otaño, I., Mandelzys, A. & Morgan, J. I. 1998 MPTP-Parkinsonism is accompanied by persistent expression of a Δ-FosB-like protein in dopaminergic pathways. *Mol. Brain Res.* **53**, 41–52. (doi:10.1016/S0169-328X(97)00269-6)

Perrotti, L. I., Hadeishi, Y., Ulery, P., Barrot, M., Monteggia, L., Duman, R. S. & Nestler, E. J. 2004 Induction of ΔFosB in reward-related brain regions after chronic stress. *J. Neurosci.* **24**, 10 594–10 602. (doi:10.1523/JNEUROSCI.2542-04.2004)

Perrotti, L. I. *et al.* 2005 ΔFosB accumulates in a GABAergic cell population in the posterior tail of the ventral tegmental area after psychostimulant treatment. *Eur. J. Neurosci.* **21**, 2817–2824. (doi:10.1111/j.1460-9568.2005.04110.x)

Perrotti, L. I. *et al.* 2008 Distinct patterns of ΔFosB induction in brain by drugs of abuse. *Synapse* **62**, 358–369. (doi:10.1002/syn.20500)

Picetti, R., Toulemonde, F., Nestler, E. J., Roberts, A. J. & Koob, G. F. 2001 Ethanol effects in ΔFosB transgenic mice. *Soc. Neurosci. Abs.* 745.16.

Pich, E. M., Pagliusi, S. R., Tessari, M., Talabot-Ayer, D., hooft van Huijsduijnen, R. & Chiamulera, C. 1997 Common neural substrates for the addictive properties of nicotine and cocaine. *Science* **275**, 83–86. (doi:10.1126/science.275.5296.83)

Renthal, W. *et al.* 2007 Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron* **56**, 517–529. (doi:10.1016/j.neuron.2007.09.032)

Renthal, W., Carle, T. L., Maze, I., Covington III, H. E., Truong, H.-T., Alibhai, I., Kumar, A., Olson, E. N. & Nestler, E. J. In press. ΔFosB mediates epigenetic desensitization of the *c-fos* gene after chronic amphetamine. *J. Neurosci.*

Robinson, T. E. & Kolb, B. 2004 Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology* **47**, S33–S46. (doi:10.1016/j.neuropharm.2004.06.025)

Russo, S. J. *et al.* 2007 NFκB signaling regulates cocaine-induced behavioral and cellular plasticity. *Soc. Neurosci. Abs.*, 611.5.

Shaffer, H. J. & Eber, G. B. 2002 Temporal progression of cocaine dependence symptoms in the US National Comorbidity Survey. *Addiction* **97**, 543–554. (doi:10.1046/j.1360-0443.2002.00114.x)

Shippenberg, T. S. & Rea, W. 1997 Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol. Biochem. Behav.* **57**, 449–455. (doi:10.1016/S0091-3057(96)00450-9)

Taylor, J. R., Lynch, W. J., Sanchez, H., Olausson, P., Nestler, E. J. & Bibb, J. A. 2007 Inhibition of Cdk5 in the nucleus accumbens enhances the locomotor activating and incentive motivational effects of cocaine. *Proc. Natl Acad. Sci. USA* **104**, 4147–4152. (doi:10.1073/pnas.0610288104)

Teegarden, S. L. & Bale, T. L. 2007 Decreases in dietary preference produce increased emotionality and risk for dietary relapse. *Biol. Psychiatry* **61**, 1021–1029. (doi:10.1016/j.biopsych.2006.09.032)

Teegarden, S. L., Nestler, E. J. & Bale, T. L. In press. ΔFosB-mediated alterations in dopamine signaling are normalized by a palatable high fat diet. *Biol. Psychiatry.*

Tsankova, N., Renthal, W., Kumar, A. & Nestler, E. J. 2007 Epigenetic regulation in psychiatric disorders. *Nat. Rev. Neurosci.* **8**, 355–367. (doi:10.1038/nrn2132)

Ulery, P. G., Rudenko, G. & Nestler, E. J. 2006 Regulation of ΔFosB stability by phosphorylation. *J. Neurosci.* **26**, 5131–5142. (doi:10.1523/JNEUROSCI.4970-05.2006)

Vialou, V. F., Steiner, M. A., Krishnan, V., Berton, O. & Nestler, E. J. 2007 Role of ΔFosB in the nucleus accumbens in chronic social defeat. *Soc. Neurosci. Abs.*, 98.3.

Wallace, D., Rios, L., Carle-Florence, T. L., Chakravarty, S., Kumar, A., Graham, D. L., Perrotti, L. I., Bolaños, C. A. & Nestler, E. J. 2007 The influence of ΔFosB in the nucleus accumbens on natural reward behavior. *Soc. Neurosci. Abs.*, 310.19.

Werme, M., Messer, C., Olson, L., Gilden, L., Thorén, P., Nestler, E. J. & Brené, S. 2002 ΔFosB regulates wheel running. *J. Neurosci.* **22**, 8133–8138.

Winstanley, C. A. *et al*. 2007 ΔFosB induction in orbitofrontal cortex mediates tolerance to cocaine-induced cognitive dysfunction. *J. Neurosci.* **27**, 10 497–10 507. (doi:10.1523/JNEUROSCI.2566-07.2007)

Yen, J., Wisdom, R. M., Tratner, I. & Verma, I. M. 1991 An alternative spliced form of FosB is a negative regulator of transcriptional activation and transformation by Fos proteins. *Proc. Natl Acad. Sci. USA* **88**, 5077–5081. (doi:10.1073/pnas.88.12.5077)

Young, S. T., Porrino, L. J. & Iadarola, M. J. 1991 Cocaine induces striatal c-fos-immunoreactive proteins via dopaminergic D1 receptors. *Proc. Natl Acad. Sci. USA* **88**, 1291–1295. (doi:10.1073/pnas.88.4.1291)

Zachariou, V. *et al*. 2006 An essential role for ΔFosB in the nucleus accumbens in morphine action. *Nat. Neurosci.* **9**, 205–211. (doi:10.1038/nn1636)