

Disorders of the Nervous System

Fosb Induction in Nucleus Accumbens by Cocaine Is Regulated by E2F3a

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Abstract

The transcription factor Δ FosB has been proposed as a molecular switch for the transition from casual, volitional drug use into a chronically addicted state, but the upstream regulatory mechanisms governing Δ FosB expression are incompletely understood. In this study, we find a novel regulatory role for the transcription factor E2F3, recently implicated in transcriptional regulation by cocaine, in controlling Δ FosB induction in the mouse nucleus accumbens (NAc) following cocaine administration. We find that an E2F consensus sequence 500 bp upstream of the *Fosb* transcription start site is enriched for E2F3 specifically over other E2F isoforms. We further conclude that Δ FosB expression is regulated specifically by E2F3a, not E2F3b, that *E2f3a* expression is specific to D1 receptor-expressing medium spiny neurons, and that E2F3a overexpression in NAc recapitulates the induction of *Fosb* and Δ *Fosb* mRNA expression observed after chronic cocaine exposure. E2F3a knockdown in NAc does not abolish Δ *Fosb* induction by cocaine, a result consistent with previously published data showing that singular knockdown of upstream regulators of Δ FosB is insufficient to block cocaine-induced expression. Finally, to elucidate potential combinatorial epigenetic mechanisms involved in E2F3a's regulation of *Fosb*, we explore H3K4me3 enrichment at the *Fosb* promoter and find that it is not enhanced by E2F3a overexpression, suggesting that it may instead be a pre-existing permissive mark allowing for E2F3a to interact with *Fosb*. Together, these findings support a role for E2F3a as a novel, upstream regulator of the addiction-mediating transcription factor Δ FosB in NAc.

Key words: addiction; cocaine; E2F3a; Δ FosB; nucleus accumbens; transcription

Significance Statement

A hallmark question in the neuroscience of addiction is how repeated drug use is translated into long-lasting changes in neural regulation. Long-lasting changes in behavior are mediated in part by intracellular transcriptional mechanisms which affect gene expression and neural circuitry. Therefore, pinpointing signaling pathways downstream of abused drugs is a high priority for understanding the addiction process. To this end, we identify and characterize E2F3a as a novel regulator of Δ FosB, a transcription factor widely studied as a molecular switch for the transition from casual drug use to addiction.

Introduction

Drug addiction is a partly heritable disorder, with ~50% of risk explained by genetic sequence variations (Meyre and Mayhew, 2017). Thus, many preclinical and clinical

studies have focused on epigenetic effects and gene expression differences induced by chronic exposure to drugs of abuse. The transcription factor Δ FosB, an alter-

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native splice variant encoded by the *Fosb* gene, accumulates in nucleus accumbens (NAc) after repeated exposure to virtually all abused substances and thus has been proposed as a molecular switch for the transition from casual drug use into the chronically addicted state by reorganizing gene expression (Nestler, 2015). This phenomenon positions Δ FosB and its encoding gene *Fosb* as unique therapeutic targets.

Numerous studies have demonstrated the necessity and sufficiency of Δ FosB for molecular and behavioral responses to cocaine and other stimulants, and have focused on downstream genes controlled by Δ FosB induction in stimulant addiction (Hiroi et al., 1997; Carlezon et al., 1998; Kelz et al., 1999; McClung and Nestler, 2003; Renthal et al., 2008, 2009; Maze et al., 2010; Grueter et al., 2013; Heller et al., 2014). However, little is known about how Δ FosB induction itself is regulated downstream of drug action. Clarifying mechanisms of Δ FosB induction in the context of stimulant addiction is critical for further understanding how this transcription factor contributes to the pathogenesis of addiction. To this end, this study uses cocaine exposure to investigate a putative transcriptional mediator of Δ FosB induction in NAc.

A prior study that used ChIP-seq and RNA-seq in parallel to investigate unbiased, genome-wide transcriptional mechanisms underlying persistent changes in gene expression in mouse NAc after repeated cocaine administration identified the E2F family of transcription factors as among the most highly implicated upstream regulator of both gene expression and alternative splicing (Feng et al., 2014). A more recent study showed that E2F3a, one of two E2F3 isoforms, mediates a substantial portion of changes in gene expression and alternative splicing in NAc after chronic cocaine exposure as well as promotes behavioral actions of the drug (Cates et al., 2018b). Given these findings, we hypothesized that E2F3a might also regulate Δ FosB expression and splicing.

Δ FosB arises via an alternative splicing event of *Fosb* mRNA in which Exon IV is truncated (Alibhai et al., 2007; Fig. 1A). Because two degradation domains are cleaved in the truncation of Exon IV, Δ FosB is unusually stable and accumulates on repeated *Fosb* induction (Nye and Nestler, 1996; Carle et al., 2007). Furthermore, phosphorylation of Ser27 in Δ FosB following cocaine exposure leads to further enhanced stability (Ulery-Reynolds et al., 2009). Although some factors leading to Δ FosB's induction and accumulation have been elucidated—polypyrimidine tract binding protein 1 (PTB; encoded by *Ptbp1*), cAMP response element binding protein (CREB), serum response

factor (SRF), and early growth response factor 3 (EGR3; Marinescu et al., 2007; Vialou et al., 2012; Chandra et al., 2015)—many of the upstream regulators and mechanisms governing Δ FosB expression following drug exposure remain to be clarified. Here, we examine the influence of E2F3a on Δ FosB expression in four brain regions related to processing the molecular and behavioral effects of cocaine: NAc, caudate–putamen (CPU), ventral tegmental area (VTA), and prefrontal cortex (PFC). Our goal is to gain insight into the regulation of this key upstream regulator of cocaine-mediated neural and behavioral changes.

E2F transcription factors are involved in chromatin modification, gene regulation, and RNA processing via direct interaction with and recruitment of histone modifying enzymes and splicing machinery, as well as binding directly to splice sites and influencing the expression of splicing factors (Lees et al., 1993; Ahlander and Bosco, 2009). E2F3 has two alternative splice forms, E2F3a and E2F3b, which have been shown to be activating or repressive under different conditions (Leone et al., 2000). Like all E2F family members, E2F3a and E2F3b share a canonical consensus sequence “TTTCGCGC” (Reichel et al., 1987; Hume et al., 2015). Here, we demonstrate that E2F3a is a key upstream regulator of *Fosb*, and induces expression of the canonical addiction-mediating transcription factor, Δ FosB. Specifically, we show that E2F3 binds at an E2F consensus sequence 500 bp upstream of the *Fosb* transcription start site (TSS) and that E2F3a, but not E2F3b, expression is increased in dopamine receptor type 1 (D1)-expressing medium spiny neurons (MSNs) following cocaine exposure. Further, overexpression of E2F3a, but not E2F3b, recapitulates induction of Δ FosB as seen with repeated cocaine administration. These data establish a novel role for E2F3a in the brain in regulating the molecular response to cocaine, specifically in regulating expression of the transcription factor Δ FosB in NAc.

Materials and Methods

Animals and treatments

C57BL/6J male mice (The Jackson Laboratory), 7–8 weeks old and weighing 25–30 g, were habituated to the animal facility 1 week before use and maintained at 22–25°C on a 12 h light/dark cycle. All animals had access to food and water *ad libitum*. All animal procedures were performed in accordance with guidelines of the Institutional Animal Care and Use Committee at the Icahn School of Medicine at Mount Sinai.

For quantitative chromatin immunoprecipitation (qChIP) assays and gene expression measures following fluorescence activated cell sorting (FACS), mice were injected intraperitoneally with saline or cocaine (20 mg/kg) once daily for 7 d in their home cage. Mice were then euthanized 24 h following the final cocaine injection.

For assaying the effects of viral-mediated E2F3a knock-down, mice were injected intraperitoneally with saline once daily for 4 d beginning 2 d post-viral infusion. On the fifth day, animals were either injected with a final dose of saline or a single, acute dose of cocaine (20 mg/kg) and euthanized 1 h later.

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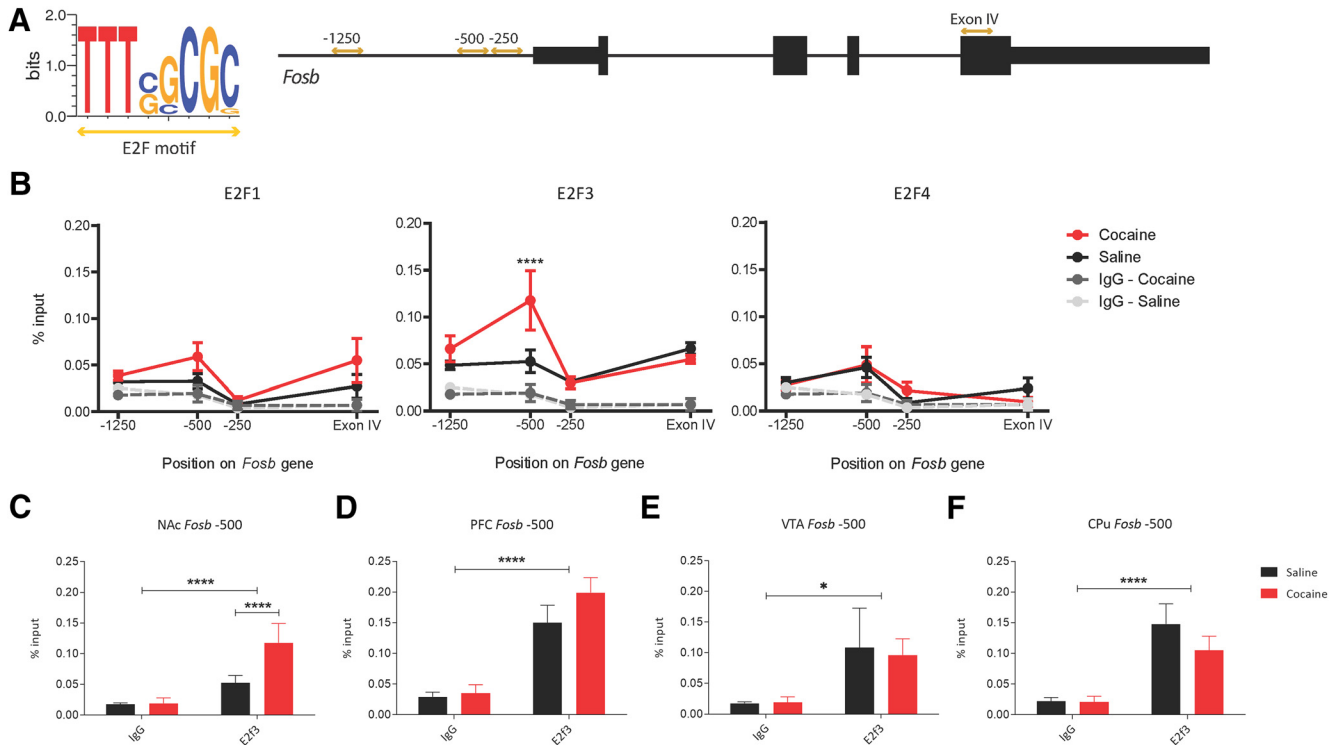


Figure 1. E2F3 binding at the *Fosb* gene promoter is regulated in NAc by cocaine. **A**, E2F consensus sequence and a schematic diagram of *Fosb*. Primer sets designed against putative E2F binding sites noted with golden double arrowheads. **B**, E2F3 binding, not E2F1 or E2F4, was increased following cocaine administration 500 bp upstream of the *Fosb* TSS (interaction of drug and gene position was observed by two-way ANOVA: $F_{(9,60)} = 13.35$, $p < 0.0001$; Cocaine vs saline at -500 bp via *post hoc* analysis: $t_{(8)} = 9.904$, $****p < 0.0001$). All data points have error bars representing \pm SEM. **C–F**, Significant binding of E2F3 over an IgG control was observed and significantly increased by cocaine in **(C)** NAc ($F_{(9,60)} = 13.35$, $****p < 0.0001$, $t_{(8)} = 9.904$, $****p < 0.0001$). Significant binding of E2F3 was observed but was not affected by cocaine administration in **(D)** PFC, binding over an IgG control ($F_{(1,16)} = 48.44$, $****p < 0.0001$), no main effect of drug observed ($F_{(1,16)} = 1.789$, $p = 0.1998$); **(E)** VTA, binding over an IgG control ($F_{(1,10)} = 7.024$, $*p = 0.0243$), no main effect of drug observed ($F_{(1,10)} = 0.029$, $p = 0.869$); or **(F)** CPU, binding over an IgG control ($F_{(1,16)} = 25.113$, $****p < 0.0001$), no main effect of drug observed ($F_{(1,16)} = 1.12$, $p = 0.305$). $N = 4–5$, with three mice pooled per sample for all qChIP assays.

Viral-mediated gene transfer

Mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and prepared for stereotaxic surgery. Thirty-three gauge syringe needles (Hamilton) were used to bilaterally infuse 0.5 μ l virus in NAc at a rate of 0.1 μ l/min at 1.6 mm anterior, 1.5 mm lateral, and 4.4 mm ventral from bregma at a 10° angle, or in PFC at 1.8 mm anterior, 0.75 mm lateral, and 2.7 mm ventral from bregma at a 15° angle. We used bicistronic p1005 HSV vectors expressing GFP alone or GFP plus E2f3a or E2f3b for overexpression experiments, or HSV vectors that expressed GFP as well as microRNAs targeted against LacZ or *E2f3a* for knockdown experiments. In these systems, GFP expression is driven by a cytomegalovirus promoter, whereas the select gene of interest is driven by the IE4/5 promoter (Maze et al., 2010). Viral expression was confirmed during tissue collection using fluorescence microscopy (Leica) to visualize GFP and confirm targeting of NAc or PFC. The effectiveness of the overexpression and knock-out vectors in NAc has been published (Cates et al., 2018b) and replicated here (data not shown).

Tissue collection

Tissue was collected at the appropriate time point following pharmacological or viral manipulation via cervical

dislocation followed by dissection of NAc with a 14-gauge blunt needle for cocaine studies, or a 15-gauge needle for viral studies to ensure only virally infected tissue was dissected. PFC was dissected with a single central 12-gauge punch for cocaine studies, or a 14-gauge punch for viral studies. Tissue was immediately frozen on dry ice. Single animal samples were used in all RNA-qPCR experiments. For qChIP experiments, animals were pooled as described below.

Quantitative chromatin immunoprecipitation

qChIP was performed on NAc or PFC punches, pooled from three mice, 72 h after viral infection, or from two mice, 24 h after the last drug treatment. qChIP methodology was modified from Ferguson et al. (2015). Chromatin was prepared by fixation in 940 μ l 1% formaldehyde for 12 min followed by addition of 64 μ l 2 M glycine to stop the fixing reaction for 5 min. Punches were then washed 5 times in cold PBS, and passed through a 21-gauge syringe 10 times to homogenize the tissue. The homogenized tissue was centrifuged at 1250 rcf, and then resuspended and incubated in a 0.5% NP-40 cellular lysis buffer for 15 min, centrifuged at 2700 rcf and resuspended in a 1% SDS nuclear lysis buffer for 10 min. The samples were then sheared using a Diagenode Bioruptor

Table 1. ChIP-qPCR Primers along the *Fosb* gene

Location on <i>Fosb</i>	Forward	Reverse
–1250	ATGGGACTCAGGTTGTCAGG	AGCCAGGGCTACACAGAGAA
–500	GAGTTGCACCTTCTCCAACC	GGCCAGTGTTTGTGGTA
–250	ATGGCTAATTGCGTCACAGG	ACCTCCCAAACCTCTCCCTTC
Exon IV	CAACCTGACGGCTTCTCT	CGGGTTTGTGGTTTGTGGTA

These qChIP primers were used to amplify three putative E2F consensus sequences in *Fosb*: 500 and 250 bp upstream of the TSS, and a site within Exon IV. A sequence 1250 bp upstream of the TSS was used as a negative control.

XL at high sonication intensity for 30 s on/30 s off for 10 min twice (to prevent overheating). Fragment size and chromatin DNA concentration was verified with an Agilent 2100 Bioanalyzer at 150–300 bp. Sheared chromatin was incubated overnight in a 0.1% SDS solution with the following antibodies previously bound to magnetic beads following the Dynabead protocol (Dynabeads M-280, Life Technologies): antibody to E2F3 [Santa Cruz Biotechnology, E2F-3 (C-18) × (sc-878X)], antibody to Rabbit-IgG [Santa Cruz Biotechnology, normal rabbit IgG × (sc-2027X)], antibody to H3K4me3 [ActiveMotif, Histone H3K4me3 antibody (pAb); 39915]. 1 μg of DNA was used for E2F3 and IgG IPs, 250 ng for H3K4me3. After incubation, beads were washed four times: (1) low salt (150 mM NaCl), (2) high salt (500 mM NaCl), (3) 150 mM LiCl, and (4) TE + 50 mM NaCl. Chromatin was eluted off the beads using a 100 mM sodium bicarbonate/1% SDS solution at 65°C for 30 min. Then reverse crosslinking took place overnight at 65°C, and DNA purification was performed via Qiagen Spin Columns. Binding to the *Fosb* gene was determined by qRT-PCR using primers detailed in Table 1. The E2F consensus sequence was identified using MotifMap (Daily et al., 2011). The binding sites investigated were determined using MatInspector, which predicts functional transcription factor binding sites using a position weight matrix approach to identify known binding motifs and compute a likelihood of actual functional binding determined by the surrounding base pair context (Cartharius et al., 2005).

FACS isolation of MSNs

Male D1-Tomato and D2-GFP were used to tag D1-receptor expressing and dopamine 2 (D2)-receptor expressing MSNs, respectively, with tdTomato or GFP fluorophores. Whole, tagged neurons were isolated and collected as previously described (Engmann et al., 2017). Briefly, NAc punches were collected in ice-cold digestion buffer, digested in papain solution, and titrated to allow for cells to gently disperse. Intact, dissociated cells were selected by passing the samples over an ovomucoid gradient. Sorting was performed on a BD FACS Aria II and collected into Trizol LS-reagent (Ambion, 10296-028) and RNA was extracted using the Direct-zol RNA miniprep kit with DNase treatment (Zymo, R2050), following the man-

ufacturer's recommendations. RNA quality was assessed with an Agilent RNA 6000 PICO kit (Agilent Technologies, 5067-1513).

RNA isolation and qPCR

RNA isolation, qPCR and data analysis were performed as previously described (Maze, et al., 2010). Briefly, RNA was isolated with TriZol reagent (Invitrogen) and was further purified with RNAeasy microkits from Qiagen. Samples were analyzed on a NanoDrop 2000 to assess RNA purity (260:280 ratio between 1.80 and 2 and 260/230 > 1). Three hundred nanograms of RNA were converted to cDNA using iScript (Bio-Rad) according to the manufacturer's protocol, and qPCR was conducted using SYBR green (Quanta) with an Applied Biosystems 7900HT RT PCR system using the following cycle parameters: 2 min at 95°C; 40 cycles of 95°C for 15 s, 59°C for 30 s, 72°C for 33 s; and graded heating to 95°C to generate dissociation curves for confirmation of single PCR products. Data were analyzed by comparing C_t values of the treatment condition to the control condition with the $\Delta\Delta C_t$ method (Tsankova et al., 2006). Primer sequences are listed in Table 2.

Statistics

All analyses were performed using Prism software (GraphPad). Student's *t* tests were used for any pairwise comparisons, and two-way ANOVAs were used for all multiple comparisons, followed by Bonferroni *post hoc* tests where appropriate. A type I error significance level of 0.05 was used to determine statistical significance for all calculations.

Results

Regulation of *Fosb* by E2F3 after cocaine administration in NAc is both isoform- and region-specific

We identified, through a detailed investigation of the *Fosb* gene using MatInspector, three putative E2F binding sites (Daily et al., 2011; Cartharius et al., 2005). Two are in the promoter ~250 and 500 bp upstream of the TSS. A third is in Exon IV near the alternative splice site where the truncation of full-length *Fosb* mRNA occurs and leads to

Table 2. RT-qPCR Primers for *Fosb* transcripts and *Gapdh* for probing mRNA expression

Transcript	Forward	Reverse
<i>Fosb</i>	GTGAGAGATTGCCAGGGTC	AGAGAGAAGCCGTCAGGTTG
Δ <i>Fosb</i>	AGGCAGAGCTGGAGTCGGAGAT	GCCGAGGACTTGAACCTCACTCG
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

These qPCR primers were used to amplify and assess expression of *Fosb* and Δ *Fosb* mRNA. *Gapdh* was used as a housekeeping gene in the $\Delta\Delta C_t$ method.

Table 3. Statistical table

Figure	Data structure	Type of test	Power
a Fig. 1B,C	Normal distribution	4 × 4 ANOVA	0.0621
b Fig. 1D	Normal distribution	2 × 2 ANOVA	0.0595
c Fig. 1E	Normal distribution	2 × 2 ANOVA	0.0595
d Fig. 1F	Normal distribution	2 × 2 ANOVA	0.0595
e Fig. 2A	Normal distribution	2 × 2 ANOVA	0.0558
f Fig. 2B	Normal distribution	2 × 2 ANOVA	0.0558
g Fig. 2D	Normal distribution	2 × 3 ANOVA	0.0692
h $\Delta F_{osb}/F_{osb}$ mRNA ratio	Normal distribution	Student's <i>t</i> test	0.0661
i Fig. 2E	Normal distribution	2 × 2 ANOVA	0.0607
j Fig. 2F	Normal distribution	2 × 4 ANOVA	0.0651
k Fig. 3A	Normal distribution	2 × 2 ANOVA	0.0607
l Fig. 3B	Normal distribution	2 × 2 ANOVA	0.0607

expression of ΔF_{osb} mRNA (Fig. 1A). To test whether *Fosb* and thus ΔF_{osb} expression is regulated by E2F3 specifically, and not another E2F isoform, we performed qChIP for E2F1, E2F3, and E2F4 in NAc under both saline and repeated cocaine conditions (once daily intraperitoneal injections for 7 d). E2F2, E2F5, E2F6, E2F7, and E2F8 are sparsely expressed in the adult mouse brain (Lein et al., 2007) and were not investigated in this study. We designed primers to amplify the three identified binding sites of interest, as well as a negative control region where no binding was expected at 1250 bp upstream of the TSS. An interaction of cocaine treatment and gene position was observed for E2F3 only ($F_{(9,60)} = 13.35$, $p < 0.0001$; $N = 4-5$, 3 mice/sample for all qChIP assays; row a, Table 3), with a specific increase in binding following cocaine administration at the site 500 bp upstream of the TSS ($t_{(8)} = 9.904$, $p < 0.0001$; Fig. 1B,C; row a, Table 3). Note that no available antibody can distinguish between E2F3a and E2F3b.

Next, to determine whether regulation of *Fosb* by E2F3 at this -500 bp site is specific to NAc, we repeated qChIP for E2F3 in three additional regions of the reward circuitry: PFC, VTA, and CPU. In PFC, significant binding over an IgG control was observed ($F_{(1,16)} = 48.44$, $p < 0.0001$; $N = 5$; row b, Table 3), but this effect was not significantly different between saline and cocaine conditions ($t_{(8)} = 1.69$, $p = 0.2208$; Fig. 1D; row b, Table 3). Modest binding was observed in VTA, but again this was not significantly different between saline and cocaine ($F_{(1,10)} = 7.024$, $p = 0.0243$; $t_{(5)} = 0.275$, $p = 0.9999$; $N = 3-4$; Fig. 1E; row c, Table 3). E2F3 binding in CPU was also significantly increased over an IgG control ($F_{(1,16)} = 25.113$, $p < 0.0001$; $N = 5$; row d, Table 3), with no significant effect of cocaine ($t_{(8)} = 1.451$, $p = 0.3324$; Fig. 1F; row d, Table 3). Together, these data indicate that regulation of E2F3 binding to *Fosb* after repeated cocaine is specific to NAc.

Expression of E2F3a, not E2F3b, is increased by cocaine in NAc D1 MSNs

To explore the cell-type-specific expression of *E2f3*'s two isoforms, E2F3a and E2F3b, we performed FACS on tagged D1 MSNs or D2 MSNs and analyzed the mRNA expression of each transcript. In isolated D1 MSNs, both *E2f3a* and *E2f3b* are expressed at baseline levels, but only *E2f3a* expression is increased by repeated cocaine expo-

sure. Main effects of both drug treatment ($F_{(1,9)} = 9.624$, $p = 0.0127$; row e, Table 3) and transcript variant ($F_{(1,9)} = 6.845$, $p = 0.028$; $N = 3-4$; row e, Table 3) were observed and a significant increase in *E2f3a* expression only was confirmed via *post hoc* analysis (*E2f3a*: $t_{(5)} = 4.186$, $p = 0.0047$; *E2f3b*: $t_{(4)} = 0.3328$, $p = 0.9999$; Fig. 2A; row e, Table 3). In D2 MSNs, *E2f3b* is expressed minimally but no *E2f3a* expression was detected under control or cocaine conditions ($F_{(1,9)} = 15.489$, $p = 0.0034$; $N = 3-4$; Fig. 2B; row f, Table 3). Importantly, these data indicate that *E2f3a* is induced by cocaine selectively in the same neuronal population in which cocaine induction of ΔF_{osb} occurs. Thus, Lobo et al. (2013) found that ΔF_{osb} is induced by cocaine in D1 but not D2 NAc MSNs, and Grueter et al. (2013) demonstrated that selective overexpression of ΔF_{osb} in D1 but not D2 NAc MSNs enhances behavioral responses to cocaine.

Overexpression of E2F3a recapitulates cocaine-induced *Fosb* and ΔF_{osb} mRNA expression

To probe which E2F3 isoform regulates ΔF_{osb} expression, we performed stereotaxic surgery to infuse HSV-GFP, -E2F3a, or -E2F3b into NAc. A main effect of virus was observed ($F_{(2,29)} = 9.332$, $p = 0.0007$; $N = 6$; row g, Table 3), and a significant E2F3a-induced increase in both *Fosb* and ΔF_{osb} mRNA expression was confirmed via *post hoc* analysis (*Fosb*: $t_{(10)} = 3.138$, $p = 0.0078$; ΔF_{osb} : $t_{(9)} = 2.868$, $p = 0.0185$; Fig. 2D; row g, Table 3). The ratio of *Fosb*- ΔF_{osb} mRNA was not affected by E2F3a overexpression ($t_{(9)} = 0.3142$, $p = 0.7605$; row h, Table 3), consistent with the lack of binding of E2F3 to the *Fosb* splice site in Exon IV. E2F3b overexpression did not affect mRNA expression of either *Fosb* isoform (*Fosb*: $t_{(10)} = 1.336$, $p = 0.5757$; ΔF_{osb} : $t_{(10)} = 0.575$, $p = 0.9999$; Fig. 2D; row g, Table 3).

Because significant binding of E2F3 was observed in PFC (Fig. 1E), but was unaffected by cocaine, we used this region as a control for the effect of E2F3a overexpression in NAc. E2F3a overexpression in PFC did not affect expression of either *Fosb* or ΔF_{osb} mRNA ($F_{(1,19)} = 1.637$, $p = 0.2162$; $N = 6$; Fig. 2E; row i, Table 3).

To further explore the necessity of E2F3a binding to *Fosb* to induce ΔF_{osb} expression, we knocked down expression of E2F3a with a miRNA and assessed ΔF_{osb} mRNA levels following an acute dose of experimenter-administered cocaine. We found a main effect of treatment ($F_{(3,20)} = 3.297$, $p = 0.0415$; $N = 5-7$; row j, Table 3), and confirmed via *post hoc* analysis that an acute dose of cocaine induces a near-significant increase in ΔF_{osb} mRNA expression (miR-LacZ + saline vs miR-LacZ + cocaine: $t_{(9)} = 2.492$, $p = 0.0508$; Fig. 2F; row j, Table 3). On E2F3a knockdown, cocaine no longer induced ΔF_{osb} (miR-LacZ + saline vs miR-E2f3a + cocaine: $t_{(9)} = 1.223$, $p = 0.6855$; row j, Table 3), but the difference between E2F3a knockdown and control conditions did not achieve statistical significance (miR-LacZ + cocaine vs miR-E2f3a + cocaine: $t_{(10)} = 1.3311$, $p = 0.9999$; Fig. 2F; row j, Table 3). Together, these findings indicate that E2F3a binding to the *Fosb* promoter is sufficient for induction of ΔF_{osb} expression but is not solely required. This is consistent with published data on two other transcription factors that regulate ΔF_{osb} : SRF and CREB. Ablation of

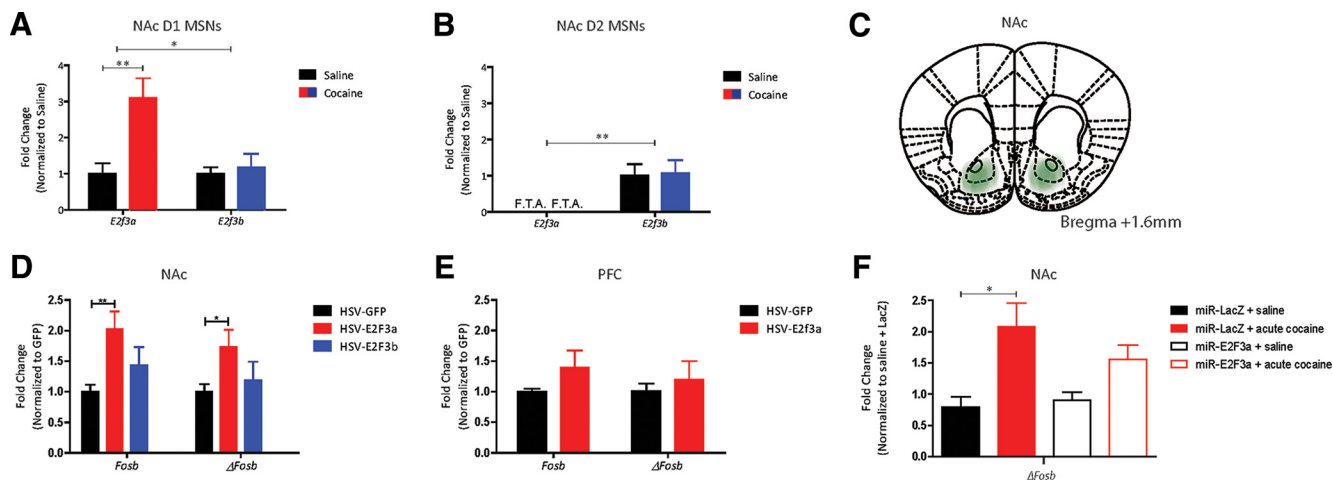


Figure 2. E2F3a is expressed in NAc D1 MSNs and overexpression of E2F3a in NAc regulates expression of *Fosb* isoforms in NAc. **A**, Expression of *E2f3a* and *E2f3b* in FACS-isolated D1 MSNs. Main effects of both drug treatment ($F_{(1,9)} = 9.624$, $p = 0.0127$) and transcript variant ($F_{(1,9)} = 6.845$, $p = 0.028$) were observed. A significant increase in *E2f3a* expression was confirmed via *post hoc* analysis (*E2f3a*: $t_{(5)} = 4.186$, $**p = 0.0047$; *E2f3b*: $t_{(4)} = 0.3328$, $p = 0.9999$). $N = 3-4$. **B**, Expression of *E2f3a* and *E2f3b* in FACS-isolated D2 MSNs. A main effect of transcript was observed ($F_{(1,9)} = 15.489$, $**p = 0.0034$) but no effect of drug treatment ($F_{(1,9)} = 0.0171$, $p = 0.8987$). $N = 3-4$. **C**, NAc cross section indicating site of injection for viral-mediated gene transfer. **D**, qPCR from mouse NAc injected with HSV-GFP, HSV-E2F3a, or HSV-E2F3b. Normalized to GFP (*Gapdh* used as housekeeping gene in $\Delta\Delta C_t$ method). A main effect of virus was observed ($F_{(2,29)} = 9.332$, $p = 0.0007$). E2F3a, but not E2F3b, increased both *Fosb* and $\Delta Fosb$ mRNA expression by *post hoc* analysis (E2F3a vs GFP-*Fosb*: $t_{(10)} = 3.138$, $**p = 0.0117$; E2F3a vs GFP- $\Delta Fosb$: $t_{(9)} = 2.868$, $*p = 0.0229$; E2F3b vs GFP-*Fosb*: $t_{(10)} = 1.336$, $p = 0.5757$; E2F3b vs GFP- $\Delta Fosb$: $t_{(10)} = 0.575$, $p = 0.9999$; $N = 8$). **E**, qPCR from mouse PFC injected with HSV-GFP or HSV-E2F3a. Normalized to GFP. No main effect of virus was observed ($F_{(1,19)} = 1.637$, $p = 0.2162$; $N = 6$). **F**, qPCR from mouse NAc injected with HSV-miR-LacZ or HSV-miR-E2F3a and treated with either saline or acute cocaine (1 injection of 20 mg/kg). Normalized to miR-LacZ + saline. A main effect of drug treatment was observed ($F_{(3,20)} = 3.297$, $p = 0.0415$) and *post hoc* analysis confirmed that acute cocaine increased $\Delta Fosb$ expression and viral knockdown of E2F3a did not significantly blunt this effect (miR-LacZ + saline vs miR-LacZ + cocaine: $t_{(9)} = 2.492$, $*p = 0.0508$, miR-LacZ + saline vs miR-E2F3a + cocaine: $t_{(9)} = 1.223$, $p = 0.9999$; miR-LacZ + cocaine vs miR-E2F3a + cocaine: $t_{(10)} = 1.331$, $p = 0.9999$; $N = 5-7$).

both of these upstream regulators simultaneously blocks the cocaine-induced increase in $\Delta Fosb$, but knockdown of either molecule alone is insufficient (Vialou et al., 2012).

Overexpression of E2F3a increases binding of E2F3 at the *Fosb* promoter but does not affect H3K4me3 enrichment

To determine whether this increase in *Fosb* expression following E2F3a overexpression is because of direct binding at the *Fosb* gene promoter in NAc, qChIP was performed following overexpression of E2F3a. An interaction of virus and gene position was observed following overexpression of E2F3a ($F_{(3,35)} = 3.619$, $p = 0.0224$; $N = 5-6$, 3 mice/sample for all qChIP assays; row k, Table 3). E2F3a overexpression elicited a specific increase in E2F3 binding 500 bp upstream of the TSS, the same site at which binding was observed following cocaine exposure, but not at the two other putative binding sites as confirmed by *post hoc* analysis ($t_{(7)} = 3.285$, $p = 0.0093$; Fig. 3A; row k, Table 3). This result, along with our initial cocaine qChIP results, confirms that the putative E2F binding site 500 bp upstream of the TSS is a functional E2F response element, at least in the NAc.

In the same genome-wide study that implicated E2F3 as a regulator of molecular responses to cocaine in the NAc, H3K4me3 enrichment was highly correlated with predicted E2F3 binding motifs, suggesting that this mark may be important in E2F3 action (Feng et al., 2014). We therefore tested whether overexpression of E2F3a in NAc

increases H3K4me3 at this site in *Fosb* or if perhaps H3K4me3 is a pre-existing mark. No effect of E2F3a overexpression was observed on H3K4me3 enrichment ($F_{(1,40)} = 0.057$, $p = 0.8126$; $N = 5-6$; row l, Table 3). However, a main effect of gene position was observed ($F_{(3,40)} = 37.61$, $p < 0.0001$; row l, Table 3), with relatively high amounts of H3K4me3 enrichment near the E2F3 binding site 500 bp upstream of the TSS, as well as 250 bp upstream of TSS (Fig. 3B), indicating that H3K4me3 may be a permissive, pre-existing mark before E2F3 regulation of *Fosb*.

Discussion

Here, we demonstrate that a specific isoform of the E2F3 transcription factor, E2F3a, is a novel regulator of the cocaine-elicited expression of the addiction-associated transcription factor $\Delta FosB$. We show that in NAc specifically, E2F3 binding at the *Fosb* promoter at a site 500 bp upstream of the TSS is increased after repeated cocaine exposure. E2F3a overexpression in this brain region increases E2F3 binding at this same site and induces transcription of both *Fosb* products, *Fosb* and $\Delta Fosb$ mRNA. Interestingly, we observed significant baseline binding of E2F3 500 bp upstream of TSS in PFC, VTA, and CPU but not regulation by cocaine. This result could point to a role for E2F3 in regulating steady-state *Fosb* expression in these reward circuitry regions, but one that is not affected by drug exposure. Further, using FACS, we determine that E2F3a's regulation of $\Delta FosB$ is

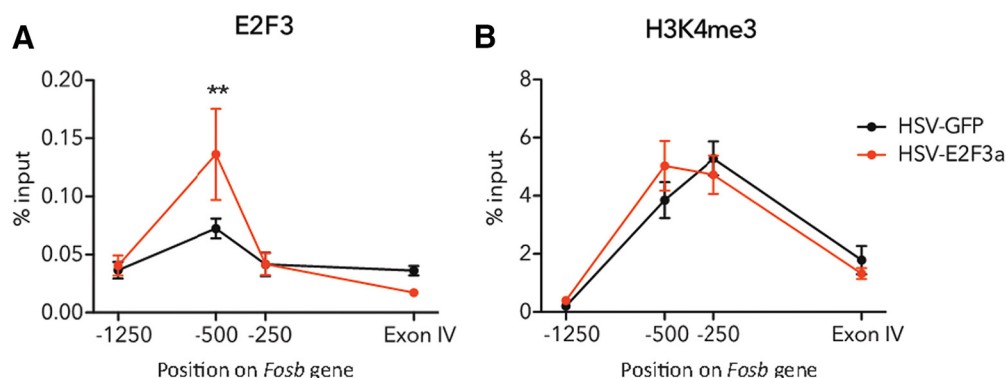


Figure 3. Viral-mediated E2F3a overexpression leads to increased E2F3 binding at *Fosb* promoter in NAc. **A**, E2F3 binding was increased at 500 bp upstream of the *Fosb* TSS after E2F3a overexpression (interaction of virus and gene position by two-way ANOVA: $F_{(3,35)} = 3.619$, $p = 0.0224$; E2F3a vs GFP at -500 bp via *post hoc* analysis: $t_{(7)} = 3.285$, $**p = 0.0093$). **B**, H3K4me3 enrichment was not affected by E2F3a overexpression (no main effect of virus observed: $F_{(1,40)} = 0.057$, $p = 0.8126$). $N = 5$ – 6 , with three mice pooled per sample for all qChIP assays.

restricted to D1 MSNs in NAc, based on *E2f3a*'s restricted baseline and cocaine-induced expression in this cell type. There was also no effect of cocaine on much lower levels of *E2f3b* in either D1 or D2 MSNs. Based on overexpression experiments, we deduced that E2F3 binding in NAc, which cannot be differentiated by qChIP because of antibody limitations, is likely E2F3a, given that overexpression of E2F3b has no effect on expression of either *Fosb* isoform, whereas E2F3a increases expression of both isoforms. By contrast, miRNA knockdown of E2F3a is not sufficient to abolish the induction of Δ FosB following cocaine. Together, these results implicate E2F3a as a novel isoform- and region-specific regulator of Δ FosB, opening a new avenue for targeting this upstream regulator of addiction-mediated behaviors and a previously unrecognized signal transduction pathway affected in NAc by cocaine exposure.

Before this investigation, two studies implicated E2F3a expression in NAc as a mediator of the transcriptional, alternative splicing, and behavioral responses to cocaine (Feng et al., 2014; Cates et al., 2018b). The first, a genome-wide study by Feng et al. (2014), included a combination of ChIP-seq and RNA-seq and identified E2F3 as one of the most prominent upstream regulators of gene expression and alternative splicing in NAc after chronic cocaine exposure. The next, a study by Cates et al. (2018b), investigated specifically which E2F3 isoform is responsible for those changes. That study found that E2F3a, not E2F3b, acts in NAc as a novel regulator of gene expression, splicing changes, and behavioral responses induced by cocaine. Because it is known that Δ FosB, like E2F3a, potentiates cocaine reward (see Introduction), these recent findings with E2F3 suggested that it might be an upstream regulator of Δ FosB.

There are three critical points of regulation in the expression of Δ FosB: transcription of *Fosb*, alternative splicing of Exon IV, and the cell-type specificity of these events. Our findings add E2F3 to a list of several other factors that influence Δ FosB expression at different points in its production. PTB (encoded by *Ptbp1*) has been shown to regulate *Fosb* splicing *in vitro* (Alibhai et al., 2007). Specifically, increased PTB leads to preferential splicing of full-length *Fosb*, but when mutated causes decreased PTB binding at Exon IV and increased prefer-

ential splicing of Δ *Fosb* (Marinescu et al., 2007). We did not find any direct involvement of E2F3a in the alternative splicing of *Fosb* and Δ *Fosb*, as there was no evidence of binding at the Exon IV alternative splice site and no effect on the ratio of the two isoforms (Fig. 1B). Interestingly, E2F3a has been shown to regulate alternative splicing of *Ptbp1* itself (Cates et al., 2018b). Thus, it is possible that cocaine leads to alternative splicing of *Ptbp1* via E2F3a, increasing expression of a specific isoform of PTB that leads to the alternative splicing event giving rise to Δ FosB. However, prior work has shown no effect of chronic cocaine on *Fosb* splicing (Alibhai et al., 2007).

The transcription factors, CREB and SRF, have previously been shown to bind the *Fosb* promoter at -250 bp in response to cocaine exposure (Renthal and Nestler, 2008; Vialou et al., 2012). Deletion of both transcription factors together ablates the cocaine-mediated induction of Δ FosB, whereas deletion of either alone has no effect. Interestingly, deletion of both CREB and SRF reduces sensitivity to cocaine reward, whereas deletion of CREB alone enhances it, suggesting that each transcription factor differentially regulates Δ FosB expression in combination with other upstream regulators. These results are in agreement with the data presented in this study, in which viral-mediated knockdown of E2F3a is insufficient to abolish the induction of Δ *Fosb* mRNA after an acute cocaine exposure (Fig. 2F).

E2F3a is expressed throughout the brain (Lein et al., 2007), and we observed significant binding of E2F3 at the -500 bp site on *Fosb* in NAc, PFC, VTA, and CPu. However, E2F3 binding to *Fosb* is specifically altered by cocaine exposure in NAc only (Fig. 1), indicating a role for E2F3 in the regulation of *Fosb* expression after cocaine exposure that is specific to NAc. Moreover, we show that expression of *E2f3a* is specific to D1 MSNs in NAc (Fig. 2A,B). Previous studies of Δ FosB have shown that it is expressed at baseline in both D1 and D2 MSNs, but that following both experimenter-administered and self-administered cocaine expression increases in D1 MSNs only (Lobo et al., 2013). These past findings align directly with our data presented here that indicate a D1 MSN-specific role for E2F3a in regulating the cocaine response in NAc.

In addition, another past study of Δ FosB's function in D1 and D2 MSNs investigated the effects of overexpression in each cell type on cocaine-elicited reward. This investigation found that Δ FosB overexpression in D1 but not D2 MSNs in NAc was sufficient to cause cocaine conditioned place preference at a dose of 3.75 mg/kg (Grueter et al., 2013). Here, we find no detectable expression of *E2f3a* in D2 MSNs in NAc at baseline, and a D1 MSN-specific increase in the transcript elicited by chronic cocaine exposure (Fig. 2A,B). Together, these results suggest that E2F3a regulates Δ FosB expression in D1 MSNs following cocaine exposure, and thus may possibly play a role in Δ FosB-controlled changes in behavior and gene expression. Given that no *E2f3a* expression was detected at baseline in D2 MSNs, how Δ FosB is regulated in this cell type remains an outstanding question. Finally, our results support regional specificity for E2F3a's function and suggest that it acts differently in distinct cell populations in different regions.

Given this MSN-specific and region-specific expression, our data support a broader understanding that ubiquitously expressed transcription factors target specific loci in different cells and specific brain regions. Despite this principle, it is interesting that we observed a non-significant decrease in E2F3 binding at the -500 bp site on *Fosb* in the CPU after cocaine exposure (Fig. 1F). NAc and CPU are differentially implicated in cocaine self-administration behaviors, with NAc being essential for the acquisition of lever-pressing and CPU being essential for the perseverance of behavior on more demanding schedules of reinforcement (Murray et al., 2012). Δ FosB is known to be induced in both regions after cocaine administration (Lobo et al., 2013). Given our findings, it would be worthwhile to investigate E2F3a's regulation of Δ FosB expression in both NAc and CPU during different stages of rodent self-administration to determine how E2F3a and Δ FosB might play a role in the transition from volitional responding to compulsive drug-seeking.

That Δ FosB expression is specifically regulated in NAc by E2F3a, and not E2F3b, is also of note. The bioinformatic prediction of E2F3 as one of the most prominent upstream regulators of cocaine-induced gene expression and alternative splicing changes suggested a novel role for the E2F family in cocaine action but did not discriminate among family members or between these two E2F3 isoforms (Feng et al., 2014). E2F3a and E2F3b share the same DNA binding, nuclear localization, and transactivation domains, but E2F3b lacks an N-terminus ubiquitin-targeting domain (He et al., 2000). In proliferating tissue, E2F3a typically acts as a gene activator and E2F3b as a repressor (Leone et al., 2000). In fact, E2F3a was recently shown to be a novel regulator of cocaine elicited reward in that its overexpression enhanced cocaine reward and locomotor behaviors as well as recapitulated cocaine-induced gene expression and splicing changes (Cates et al., 2018b). Another recent study demonstrated a role for E2F3b in regulating cocaine action in the PFC (Cates et al., 2018a). Given these findings, our data on Δ FosB expression in post-mitotic brain tissue is consistent with divergent transcriptional regulatory roles for these two E2F3 isoforms.

In addition to predicting E2F3 as a novel upstream regulator, Feng et al. (2014) found significant enrichment of H3K4me3, a histone modification associated with active promoters in euchromatin, associated with E2F binding motifs in NAc in response to chronic cocaine administration. This finding suggests that, in response to chronic cocaine, H3K4me3 in NAc is either deposited as a chromatin mark that could permit E2F3 binding or that H3K4me3 is recruited by E2F3 in concert with its cocaine response to activate gene promoters. We tested these possibilities by overexpressing E2F3a in NAc and found that H3K4me3 enrichment at the *Fosb* promoter was unaffected. This result is consistent with the former hypothesis, that H3K4me3 is a pre-existing mark in NAc neuronal euchromatin that could allow E2F3a to bind its consensus sequence 500 bp upstream of the *Fosb* TSS and induce transcription. It is interesting to note that H3K4me3 is also enriched at baseline at -250 bp (Fig. 3B), the site where CREB and SRF bind their response elements to induce *Fosb* (Vialou et al., 2012); this implicates H3K4me3's general involvement in *Fosb* transcription, though this speculation deserves further investigation in follow-up studies of *Fosb*'s transcriptional activators.

We did not investigate any other permissive or repressive chromatin marks that might interact with E2F3a at *Fosb* in NAc, such as H3K4me1, H3K27me3, or H3K27ac, among many others. Likewise, we did not investigate the status of H3K4me3 enrichment at *Fosb*'s -500 bp site in other brain reward regions. To this point, however, histone deposition data from the Roadmap Epigenomics Consortium shows enrichment of H3K4me3 at the -500 bp site upstream of the *Fosb* promoter in adult mouse forebrain, hindbrain, and midbrain (Zhou et al., 2011). This observation across brain regions is consistent with our observation that the mark is also enriched at this site under both baseline and chronic cocaine conditions in the NAc. This analysis indicates that the presence of H3K4me3 alone cannot explain why E2F3a can bind to and activate *Fosb* in NAc but not in the other brain regions studied. More targeted investigations are therefore needed to identify the histone modifications and perhaps other chromatin-remodeling factors that are responsible for E2F3a's unique action on *Fosb* expression within D1 MSNs of the NAc.

In summary, we demonstrate a novel role for E2F3a, a specific isoform of the E2F family of transcription factors, in regulating the expression of Δ FosB downstream of cocaine action in NAc. Δ FosB is a critical regulator of the behavioral responses to diverse substances of abuse that can lead to addiction. Thus, understanding how Δ FosB is regulated by upstream transcription factors like E2F3a in the context of cocaine exposure will lead to a greater understanding of how this critical regulator might be exploited for potential therapeutic development in the treatment of addiction and other disorders related to the use of commonly abused substances.

References

- Ahlander J, Bosco G (2009) The RB/E2F pathway and regulation of RNA processing. *Biochem Biophys Res Commun* 384:280–283.
- Alibhai IN, Green TA, Potashkin JA, Nestler EJ (2007) Regulation of *fosB* and Δ *fosB* mRNA expression: *in vivo* and *in vitro* studies. *Brain Res* 1143:22–33.
- Carle TL, Ohnishi YN, Ohnishi YH, Alibhai IN, Wilkinson MB, Kumar A, Nestler EJ (2007) Proteasome-dependent and -independent

- mechanisms for FosB destabilization: identification of FosB de-gon domains and implications for Δ FosB stability. *Eur J Neurosci* 25:3009–3019.
- Carlezon WA, Thome J, Olson VG, Lane-Ladd SB, Brodtkin ES, Hiroi N, Duman RS, Neve RL, Nestler EJ (1998) Regulation of cocaine reward by CREB. *Science* 282:2272–2275.
- Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, Werner T (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. *Bioinformatics* 21:2933–2942.
- Cates HM, Bagot RC, Heller EA, Purushothaman I, Lardner CK, Walker DM, Peña CJ, Neve RL, Shen L, Nestler EJ (2018a) A novel role for E2F3b in regulating cocaine action in the prefrontal cortex. *Neuropsychopharmacology* 44:776–784.
- Cates HM, Heller EA, Lardner CK, Purushothaman I, Peña CJ, Walker DM, Cahill ME, Neve RL, Shen L, Bagot RC, Nestler EJ (2018b) Transcription factor E2F3a in nucleus accumbens affects cocaine action via transcription and alternative splicing. *Biol Psychiatry* 84:167–179.
- Chandra R, Francis CT, Konkalmatt P, Amgalan A, Gancarz AM, Dietz DM, Lobo MK (2015) Opposing role for Egr3 in nucleus accumbens cell subtypes in cocaine action. *J Neurosci* 35:7927–7937.
- Daily K, Patel VR, Rigor P, Xie X, Baldi P (2011) MotifMap: integrative genome-wide maps of regulatory motif sites for model species. *BMC Bioinformatics* 12:495.
- Engmann O, Labonté B, Mitchell A, Bashtrykov P, Calipari ES, Rosenbluh C, Loh YE, Walker DM, Burek D, Hamilton PJ, Issler O, Neve RL, Turecki G, Hurd Y, Chess A, Shen L, Mansuy I, Jeltsch A, Akbarian S, Nestler EJ (2017) Cocaine-induced chromatin modifications associate with increased expression and three-dimensional looping of *Auts2*. *Biol Psychiatry* 82:794–805.
- Feng J, Wilkinson M, Liu X, Purushothaman I, Ferguson D, Vialou V, Maze I, Shao N, Kennedy P, Koo J, Dias C, Laitman B, Stockman V, LaPlant Q, Cahill ME, Nestler EJ, Shen L (2014) Chronic cocaine-regulated epigenomic changes in mouse nucleus accumbens. *Genome Biol* 15:R65.
- Ferguson D, Shao N, Heller EA, Feng J, Neve RL, Kim HD, Call T, Magazu S, Shen L, Nestler EJ (2015) SIRT1-FOXO3a regulate cocaine actions in nucleus accumbens. *J Neurosci* 35:3100–3111.
- Grueter BA, Robison AJ, Neve RL, Nestler EJ, Malenka RC (2013) Δ FosB differentially modulates nucleus accumbens direct and indirect pathway function. *Proc Natl Acad Sci* 110:1923–1928.
- Heller EA, Cates HM, Peña CJ, Sun H, Shao N, Feng J, Golden SA, Herman JP, Walsh JJ, Mazei-Robison M, Ferguson D, Knight S, Gerber MA, Nievera C, Han MH, Russo SJ, Tamminga CS, Neve RL, Shen L, Zhang HS, et al. (2014) Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors. *Nat Neurosci* 17:1720–1727.
- He Y, Armanious MK, Thomas MJ, Cress DW (2000) Identification of E2F-3B, an alternative form of E2F-3 lacking a conserved N-terminal region. *Oncogene* 19:3422–3433.
- Hiroi N, Brown JR, Haile CN, Ye H, Greenberg ME, Nestler EJ (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 U S A* 94:10397–10402.
- Hume MA, Barrera LA, Gisselbrecht SS, Bulyk ML (2015) UniPROBE, update 2015: new tools and content for the online database of protein-binding microarray data on protein-DNA interactions. *Nucleic Acids Res* 43:D117–D122.
- Kelz MB, Chen J, Carlezon WA, Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang YJ, Marotti L, Self DW, Tkatch T, Baranaukas G, Surmeier JD, Neve RL, Duman RS, Picciotto MR, Nestler EJ (1999) Expression of the transcription factor Δ FosB in the brain controls sensitivity to cocaine. *Nature* 401:272–276.
- Lees J, Ito Vidal M, Valentine M, Look T, Harlow E, Dyson N, Helin K (1993) The retinoblastoma protein binds to a family of E2F transcription factors. *Mol Cell Biol* 13:7813–7825.
- Lein ES, et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. *Nature* 445:168–176.
- Leone G, Nuckolls F, Ishida S, Adams M, Sears R, Jakoi L, Miron A, Nevins JR (2000) Identification of a novel E2F3 product suggests a mechanism for determining specificity of repression by Rb proteins. *Mol Cell Biol* 20:3626–3632.
- Lobo MK, et al. (2013) Δ FosB Induction in striatal medium spiny neuron subtypes in response to chronic pharmacological, emotional, and optogenetic stimuli. *J Neurosci* 33:18381–18395.
- Marinescu V, Loomis PA, Ehmann S, Beales M, Potashkin JA (2007) Regulation of retention of FosB intron 4 by PTB. *PLoS One* 2:e828.
- Maze I, Covington HE, Dietz D, LaPlant Q, Renthal W, Russo SJ, Mechanic M, Mouzon E, Neve RL, Haggarty SJ, Ren Y, Sampath SC, Hurd YL, Greengard P, Tarakhovskiy A, Schaefer A, Nestler EJ (2010) Essential role of the histone methyltransferase G9a in cocaine-induced plasticity. *Science* 327:213–216.
- McClung CA, Nestler EJ (2003) Regulation of gene expression and cocaine reward by CREB and Δ FosB. *Nat Neurosci* 6:1208–1215.
- Meyre D, Mayhew A (2017) Assessing the heritability of complex traits in humans: methodological challenges and opportunities. *Curr Genomics* 18:332–340.
- Murray JE, Belin D, Everitt BJ (2012) Double dissociation of the dorsomedial and dorsolateral striatal control over the acquisition and performance of cocaine seeking. *Neuropsychopharmacology* 37:2456–2466.
- Nestler EJ (2015) Δ FosB: a transcriptional regulator of stress and antidepressant responses. *Eur J Pharmacol* 753:66–72.
- Nye HE, Nestler EJ (1996) Induction of chronic Fos-related antigens in rat brain by chronic morphine administration. *Mol Pharmacol* 49:636–645.
- Reichel R, Kovacs I, Nevins JR (1987) Developmental control of a promoter-specific factor that is also regulated by the E1A gene product. *Cell* 48:501–506.
- Renthal W, Carle TL, Maze I, Covington HE, Truong H-T, Alibhai I, Kumar A, Montgomery RL, Olson EN, Nestler EJ (2008) Delta FosB mediates epigenetic desensitization of the *c-fos* gene after chronic amphetamine exposure. *J Neurosci* 28:7344–7349.
- Renthal W, Kumar A, Xiao G, Wilkinson M, Covington HE, Maze I, Sikder D, Robison AJ, LaPlant Q, Eitz D, Russo SJ, Vialou V, Chakravarty S, Kodadek TJ, Stack A, Kabbaj M, Nestler EJ (2009) Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. *Neuron* 62:335–348.
- Renthal W, Nestler EJ (2008) Epigenetic mechanisms in drug addiction. *Trends Mol Med* 14:341–350.
- Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat Neurosci* 9:519–525.
- Ulery-Reynolds PG, Castillo MA, Vialou V, Russo SJ, Nestler EJ (2009) Phosphorylation of Δ FosB mediates its stability *in vivo*. *Neuroscience* 158:369–372.
- Vialou V, Feng J, Robison AJ, Ku SM, Ferguson D, Scobie KN, Mazei-Robison MS, Mouzon E, Nestler EJ (2012) Serum response factor and cAMP response element binding protein are both required for cocaine induction of Δ FosB. *J Neurosci* 32:7577–7584.
- Zhou X, Maricque B, Xie M, Li D, Sundaram V, Martin EA, Koebe BC, Nielsen C, Hirst M, Farnham P, Kuhn RM, Zhu J, Smirnov I, Kent WJ, Haussler D, Madden PA, Costello JF, Wang T (2011) The Human Epigenome Browser at Washington University. *Nat Methods* 12:989–990.