FosB differentially modulates nucleus accumbens direct and indirect pathway function

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ΔFosB differentially modulates nucleus accumbens direct and indirect pathway function

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Synaptic modifications in nucleus accumbens (NAc) medium spiny neurons (MSNs) play a key role in adaptive and pathological reward-dependent learning, including maladaptive responses involved in drug addiction. NAC MSNs participate in two parallel circuits, direct and indirect pathways that subserve distinct behavioral functions. Modification of NAC MSN synapses may occur in part via changes in the transcriptional potential of certain genes in a cell type–specific manner. The transcription factor ΔFosB is one of the key proteins implicated in the gene expression changes in NAc caused by drugs of abuse, yet its effects on synaptic function in NAC MSNs are unknown. Here, we demonstrate that overexpression of ΔFosB decreased excitatory synaptic strength and likely increased silent synapses onto D1 dopamine receptor–expressing direct pathway MSNs in both the NAc shell and core. In contrast, ΔFosB likely decreased silent synapses onto NAc shell, but not core, D2 dopamine receptor–expressing indirect pathway MSNs. Analysis of NAC MSN dendritic spine morphology revealed that ΔFosB increased the density of immature spines in D1 direct but not D2 indirect pathway MSNs. To determine the behavioral consequences of cell type–specific actions of ΔFosB, we selectively overexpressed ΔFosB in D1 direct or D2 indirect MSNs in NAc in vivo and found that direct (but not indirect) pathway MSN expression enhances behavioral responses to cocaine. These results reveal that ΔFosB in NAC differentially modulates synaptic properties and reward-related behaviors in a cell type- and subregion-specific fashion.

The nucleus accumbens (NAc) is a key substrate for integrating motivational information for the purpose of regulating goal-directed behavior. More than 90% of the cells within the NAc are medium spiny neurons (MSNs), which can be divided into two major subpopulations. Direct pathway MSNs, which predominantly express D1 dopamine receptors (D1 MSNs), project primarily to midbrain dopamine (DA) nuclei, whereas indirect pathway MSNs, which predominantly express D2 receptors (D2 MSNs), project mostly to the ventral pallidum, thereby indirectly influencing DA neurons (1, 2). The activity of NAc MSNs is driven chiefly by excitatory inputs from the prefrontal cortex, hippocampus, and amygdala. It has been suggested that pathology activity at NAc excitatory synapses induced by behavioral experiences such as exposure to drugs of abuse induces a reorganization of both transcriptional machinery and synapses on NAc MSNs, which in turn mediate long-lasting behavioral adaptations associated with addiction (1–3).

Recent studies have demonstrated that D1 MSNs and D2 MSNs in the core subregion of NAc exhibit different electrophysiological and synaptic properties (4) and that the two subtypes of MSNs play distinct roles in addiction-related behavior (5). However, the molecular mechanisms underlying these differences remain poorly understood. Over the last two decades, increasing evidence has linked induction of ΔFosB, a Fos family transcription factor, in NAc to changes in the brain’s reward circuitry associated with addictive- and depressive-like behaviors (3). ΔFosB, a particularly stable product of the FosB gene (6), heterodimerizes with Jun family proteins to form activator protein-1 (AP-1) complexes that bind to AP-1 sites within gene promoters to regulate transcription.

Although many Fos family proteins are transiently induced by acute drug exposure, chronic administration of virtually any drug of abuse induces the long-lasting accumulation of ΔFosB in the NAc (6). Consistent with the functional importance of this accumulation, long-term overexpression of ΔFosB selectively in D1 MSNs of the NAc and dorsal striatum of inducible bitransgenic mice causes increased locomotor responses to cocaine (7), increased conditioned place preference to both cocaine and morphine (7, 8), and enhanced cocaine self-administration (9).

The neural adaptations underlying addiction phenotypes, however, may begin immediately on initial exposure to drugs of abuse or other stimuli such as stress. In support of this hypothesis, even brief preexposure to stress causes increased propensity for drug-taking (cross-sensitization) (10–12) and vice versa (13). In addition, although the effects of stress and cocaine on the synaptic properties of these neurons have been studied (1–3, 14–16), direct evidence for the effects of ΔFosB on MSN synaptic properties is lacking. ΔFosB regulation of synaptic function is of particular interest because ΔFosB overexpression alters NAc dendritic spine morphology (17, 18), and recent work indicates that other transcriptional regulators can influence NAc MSN physiology (19) and synaptic structure (20).

To examine the shorter-term synaptic effects of ΔFosB expression in direct and indirect pathway NAc MSNs, we used bacterial artificial chromosome (BAC) transgenic mice that selectively label D1 MSNs and viral vectors that selectively target these MSN subpopulations. Targeted recordings from MSNs revealed striking cell type– and region-specific effects of ΔFosB overexpression on the properties of excitatory synapses onto NAc MSNs. Expression of ΔFosB in the NAc also modified dendritic spine morphology and addiction-related behaviors in a cell type–specific manner. These results provide further evidence that the regulation of ΔFosB levels by drugs of abuse causes cell type–specific changes in the NAc, which importantly contribute to the complex circuit adaptations underlying addiction-related behaviors.

Results

ΔFosB Does Not Affect Presynaptic Function in NAc MSNs. To examine the synaptic effects of ΔFosB overexpression in NAc MSNs, we stereotaxically injected a Herpes simplex virus (HSV) expressing ΔFosB fused to EGFP into the NAc of 8- to 10-wk-old male BAC transgenic mice in which tdTomato expression was driven by the D1 dopamine receptor promoter (21). Three to 4 d later, we made whole-cell patch-clamp recordings from NAc shell and core MSNs with the presence or absence of tdTomato defining


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D1 MSNs and D2 MSNs, respectively (4), and EGFP expression defining cells in which ΔFosB was expressed. Although it is not possible to selectively target the NAc core and shell with viruses in mice, it was important to distinguish these brain regions because of known differences in their regulation of reward- and addiction-related behaviors (2, 3). Two standard electrophysiology assays revealed that ΔFosB overexpression did not detectably influence presynaptic function in any of the NAc MSN subpopulations examined. Specifically, paired-pulse ratios (PPRs) of excitatory postsynaptic currents (EPSCs) at five different interstimulus intervals, a standard measure of transmitter release probability, were unaffected by ΔFosB overexpression in either the NAc shell or core (Fig. S1). Furthermore, the average frequency of miniature EPSCs (mEPSCs), which also correlates with presynaptic function, was unaffected by ΔFosB overexpression in D1 and D2 MSNs in both the NAc shell and core (Fig. S1).

Effects of ΔFosB on Excitatory Postsynaptic Properties in NAc D1 MSNs. To examine the effects of shorter-term ΔFosB overexpression on postsynaptic properties of excitatory synapses in NAc shell and core MSNs, we measured the ratio of AMPA receptor (AMPAR)- to NMDA receptor (NMDAR)-mediated EPSCs (22). In both the shell and core subregions, AMPAR/NMDAR ratios were significantly smaller in D1 MSNs overexpressing ΔFosB relative to neighboring uninfected D1 MSNs (Fig. 1A and B). This decrease in AMPAR/NMDAR ratios was

![Fig. 1](image-url)

Fig. 1. Overexpression of ΔFosB in NAC modifies D1 MSN synaptic strength in shell and core. (A) Representative EPSCs (A1) recorded at -70 and +40 mV from a NAc shell D1 control (black) and ΔFosB+ (red) MSN and summary graph (A2) of the ratio of the AMPAR EPSC over the NMDAR EPSC for each control (n = 7) and ΔFosB+ (n = 7) D1 MSN. Bar graphs in this and all subsequent figures show mean ± SEM (calibration bars for evoked EPSCs in this panel and all subsequent figures are 50 pA/25 ms). (B) Same as A for D1 MSNs in NAc core (n = 7 cells in each group). (C and D) Sample mEPSCs from NAc shell (C1) and core (D1) control (black) and ΔFosB+ (red) D1 MSNs (calibration bars for mEPSCs in this panel and all subsequent figures are 20 pA/1 s). Cumulative probability plots comparing mEPSC amplitudes recorded from control and ΔFosB+ D1 MSNs in NAc shell (C2 and D2). Bar graphs show average mEPSC amplitudes in these cell populations (C3 and D3) (n = 5 cells in each group). (E and F) Summary of normalized AMPAR EPSC amplitudes as a function of membrane potential recorded from control and ΔFosB+ D1 MSNs in NAc shell (E1, n = 5 cells in each group) and core (F1, n = 4 cells in each group). Bar graphs show average rectification indices for these cells (E2 and F2). (G and H) Representative EPSCs at +40 mV for control (black) and ΔFosB+ (red) D1 MSNs in NAc shell (G1) and core (H1). Bar graphs showing time to half-peak amplitude of EPSCs at +40 mV recorded from these cells (G2 and H2, n = 7 cells in each group). *P < 0.05 in this and all subsequent figures.
due, at least in part, to a decrease in AMPAR-mediated synaptic transmission, because the average amplitudes of AMPAR-mediated mEPSCs were also significantly decreased by ∆FosB overexpression (Fig. 1 C and D). Because GluA2-lacking, inwardly rectifying AMPARs are incorporated into NAc MSN synapses following withdrawal from self-administration of drugs of abuse and chronic stress protocols (14, 15, 23), we next examined the current-voltage (I-V) relationship of pharmacologically isolated AMPAR EPSCs to determine whether ∆FosB overexpression influenced the stoichiometry of synaptic AMPARs. AMPAR EPSC I-V curves recorded from both control and ∆FosB-expressing D1 MSNs in the NAc shell and core were linear and exhibited minimal inward rectification (Fig. 1 E and F). Thus, ∆FosB overexpression does not result in incorporation of GluA2-lacking AMPARs into NAc D1 MSN synapses. Finally, to determine whether NMDAR stoichiometry was affected by ∆FosB overexpression, we measured NMDAR EPSC decay time courses, which are prolonged by the presence of GluN2B subunits. ∆FosB expression caused a significantly greater time to half-peak in D1 MSNs in the NAc shell (Fig. 1 G1 and G2), and a similar trend was observed in the NAc core (Fig. 1 H1 and H2). These results suggest that similar to the expression of constitutively active CREB in NAc (24), ∆FosB overexpression elicits an increase in the proportion of GluN2B-containing NMDARs at NAc D1 MSN synapses.

Effects of ∆FosB on Excitatory Postsynaptic Properties in NAc D2 MSNs. In contrast to D1 NAc MSNs, ∆FosB overexpression in D2 MSNs elicited an increase in AMPAR/NMDAR ratios in D2 MSNs elicited an increase in AMPAR/NMDAR ratios in the

![Fig. 2](image-url)
NAC shell (Fig. 2A), but surprisingly, no detectable effect in the NAC core (Fig. 2B). Furthermore, none of the other synaptic assays revealed effects of ∆FosB overexpression in NAC D2 MSNs. AMPAR-mediated mEPSC amplitudes (Fig. 2 C and D), AMPAR EPSC I-V curves, and rectification indexes (Fig. 2 E and F), as well as NMDAR EPSC decay time courses, were all unaffected by ∆FosB overexpression in NAC D2 MSNs. These findings demonstrate that ∆FosB overexpression modifies the properties of excitatory synapses on NAC D2 MSNs to a much lesser extent than excitatory synapses on NAC D1 MSNs.

**Effects of ∆FosB on Silent Synapses in NAC D1 and D2 MSNs.** Post-synaptic silent synapses are synapses with undetectable levels of AMPARs but readily detectable NMDARs (25–27). They provide an ideal substrate for long-term potentiation (LTP) (28) and can be generated by expression of constitutively active CREB in hippocampal CA1 pyramidal cells (28) and NAC MSNs (24). Because several of the observed synaptic effects of ∆FosB overexpression may be explained by changes in the proportion of silent synapses on NAC MSNs, we performed a CV analysis of AMPAR EPSCs relative to NMDAR EPSCs. In recordings from single cells, the ratio of 1/CV2 of NMDAR EPSCs to 1/CV2 of AMPAR EPSCs directly correlates with the proportion of silent synapses (28, 29). ∆FosB overexpression caused a significant increase in this ratio in D1 MSNs in the NAC shell and core (Fig. 3 A and B). In contrast, no change in this ratio was observed in ∆FosB-expressing NAC core D2 MSNs, whereas a decrease occurred in NAC shell D2 MSNs overexpressing ∆FosB (Fig. 3C). These results are consistent with the hypothesis that ∆FosB overexpression causes an increase in the proportion of silent synapses on NAC shell and core D1 MSNs and an unsilencing of synapses on NAC shell D2 MSNs.

**Effects of ∆FosB on NACMSN Dendritic Spines.** A well-established consequence of cocaine administration in NAC MSNs is induction of dendritic spines (30, 31), a structural change that may be selective for D1 MSNs (32) and is dependent on ∆FosB (17, 18). These observations suggest that regulation of spine formation by ∆FosB may be specific to D1 MSNs. Because available mouse lines expressing fluorescent proteins with cell type specificity are not adequate for identification of dendritic spines on specific cells, to address this topic, we used a unique HSV vector that expresses mCherry in a Cre recombinase–dependent manner (Fig. S2; SI Methods) in combination with transgenic mouse lines that express Cre specifically in D1 or D2 MSNs (5). Cotransducing the NAC shell of these mice with the Cre-dependent HSV-mCherry and HSV-GFP-∆FosB (or HSV-GFP as a control) revealed that ∆FosB increases dendritic spine density in D1 MSNs but not D2 MSNs (Fig. 4). This increase is driven primarily by induction of stubby and perhaps thin spines, which are considered “immature” (33). In contrast, ∆FosB overexpression had no effect on the density of more mature mushroom-shaped dendritic spines. These ∆FosB-induced increases in immature dendritic spines in NAC D1 MSNs correlate well with the proposal that ∆FosB expression results in an increase in silent synapses (27).

**Effects of Selective ∆FosB Expression in NAC MSNs on Addiction-Related Behaviors.** To determine whether the cell type–specific effects of shorter-term ∆FosB overexpression on synaptic properties correlates with cell type–specific effects on addiction-related behaviors, we used D1-Cre and D2-Cre mice, in combination with a unique HSV vector that contains a stop codon surrounded by loxp sites (LS1) to prevent target gene expression in any cell that does not express Cre (Fig. S3). Adult male D1- and D2-Cre mice were injected bilaterally in the NAC with either HSV-GFP-LS1-∆FosB or HSV-GFP (control), and behaviors were assessed over the 5 d following surgery when transgene expression is maximal. Overexpression of ∆FosB specifically in D1 NAC MSNs caused an increase in locomotor sensitization to cocaine at both low (3.75 mg/kg) and high (7.5 mg/kg) doses, as well as an increase in the initial locomotor response to the lower dose of cocaine (Fig. 5 A and C). In contrast, ∆FosB overexpression in D2 NAC MSNs had no effect on animals’ locomotor responses to cocaine (Fig. 5 B and D). Similar results were obtained when cocaine-conditioned place preference (CPP) was assayed. D1- or D2-Cre mice injected with HSV-GFP displayed a typical dose dependence of cocaine CPP with no significant preference observed at the lower dose (Fig. 5 E and F). Overexpression of ∆FosB in D1 MSNs dramatically enhanced cocaine CPP with maximal responses observed at the lower cocaine dose (Fig. 5E), whereas ∆FosB expression in D2 NAC MSNs had no effect on cocaine reward (Fig. 5F). These results demonstrate that ∆FosB overexpression in D1 MSNs, but not in D2 MSNs, in the NAC enhances behavioral responses to cocaine.

**Discussion**

Previous work has suggested a strong link between changes in the brain’s reward circuitry due to drug-induced induction of ∆FosB and addiction-associated behaviors (6). Because ∆FosB is unusually stable and is strongly induced by chronic exposure to drugs, an effect seen in human addicts as well (18), most studies have focused on the neural and behavioral adaptations caused by longer-term ∆FosB expression, typically 2–8 wk (3, 6, 7). Nevertheless, initial exposure to drugs of abuse induces ∆FosB mRNA and protein (3, 6, 34), yet the effects of this induction on NAC MSN synaptic properties and subsequent behavioral responses to drug administration have not been explored. Here we present evidence that short-term expression of ∆FosB produces profoundly different synaptic and behavioral effects when expressed in NAC D1 versus D2 MSNs. In D1 MSNs in both the NAC core and shell, ∆FosB overexpression caused a decrease in AMPAR-mediated synaptic transmission without detectable changes in the stoichiometry of synaptic AMPARs. NMDAR-mediated EPSCs were also prolonged in NAC shell neurons (with a similar trend in core neurons), suggesting an increase in the proportion of GluN2B-containing synaptic ...
NMDARs. These changes in excitatory synapse properties can be explained, in part, by ΔFosB overexpression causing an increase in the proportion of so-called postsynaptic silent synapses. Consistent with this hypothesis, the ratio of $1/CV^2$ of NMDAR EPSCs to $1/CV^2$ of AMPAR EPSCs was increased by ΔFosB as was the density of immature spines. In contrast, ΔFosB overexpression in D2 NAc MSNs caused a relative increase in AMPAR-mediated synaptic transmission in the NAc shell but not the core. A $1/CV^2$ analysis suggests that this may be due, in part, to a decrease in the proportion of silent synapses, although we cannot rule out that a relative decrease in NMDAR-mediated synaptic transmission occurred.

To determine whether these cell type-specific synaptic adaptations in the NAc due to short-term ΔFosB overexpression correlate with any behavioral changes, we developed a unique method to restrict ΔFosB expression to either D1 or D2 NAc MSNs using the corresponding Cre driver lines. Previous work demonstrated that long-term (>6 wk) overexpression of ΔFosB in D1 direct pathway but not D2 indirect pathway MSNs in both the NAc and dorsal striatum causes increased locomotor sensitization to cocaine, as well as increased cocaine CPP at a low, but not higher, dose of the drug (7). Here we show that only 2–4 d of ΔFosB overexpression in D1 but not D2 MSNs in the NAc selectively elicits an increased locomotor response to a low cocaine dose and increased sensitization of the locomotor response to a higher dose. Similarly, this short-term ΔFosB overexpression in D1 NAc MSNs caused an increase in cocaine CPP to a lower cocaine dose. These results demonstrate that an increase in levels of ΔFosB in D1 NAc MSNs per se enhances an animal’s sensitivity to the locomotor-activating and rewarding effects of cocaine much more rapidly than previously envisioned. These results also suggest that the induction of ΔFosB caused by initial exposure to drugs may contribute to the subsequent changes that occur in responses to repeated administration.

Previous work in rats demonstrated that short-term (1–2 d) withdrawal from chronic noncontingent cocaine administration (5 d) causes the generation of silent synapses in NAc MSNs (35). This cocaine-induced synaptic modification is blocked by expression of a dominant negative form of CREB and is mimicked by expression of constitutively active CREB (24), which also induces silent synapses in the hippocampus (28). Furthermore, the silent synapses in NAc contain a greater proportion of GluN2B-containing NMDARs, blockade of which prevents the cocaine induction of silent synapses, as well as cocaine-elicited locomotor sensitization (24, 35). The synaptic effects of short-term ΔFosB overexpression demonstrated here combined with the fact that cocaine administration strongly induces ΔFosB in mice (7) suggest that cocaine administration also generates silent synapses in NAc MSNs in mice, albeit in a cell type–specific manner. Whether induction of both transcription factors is necessary for these drug-induced synaptic changes will require further study. The observation that CREB and ΔFosB both can induce silent synapses in NAc MSNs is interesting in light of substantial evidence that they mediate opposite behavioral phenotypes; whereas long-term ΔFosB promotes cocaine reward, CREB exerts the opposite effect (3, 6). However, short-term ΔFosB overexpression in inducible bitransgenic mice dampened cocaine’s behavioral effects (36), a result that may reflect lower levels of ΔFosB induced at this time point or the direct consequences of the shorter periods of expression. The findings presented here suggest the former possibility because short periods of high levels of HSV-mediated expression of ΔFosB in D1 NAc MSNs, like longer-term expression, enhanced the behavioral effects of cocaine. Clearly, further work is needed to understand the paradoxical effects of the induction of CREB versus ΔFosB in the NAc. One explanation may be the cell type specificity of their actions, because cocaine induction of ΔFosB is selective for D1 MSNs, whereas...
CREB induction occurs equally in D1 and D2 MSNs, as well as in some GABAergic interneurons (3). It will also be critical to identify the numerous target genes through which these transcription factors induce their electrophysiological and behavioral effects.

The NAc is a complex brain region, consisting of a heterogeneous mix of several cell types with distinct roles in several interrelated circuits and behavioral outputs (1, 3). Here, we focus specifically on MSNs, because they are the projection neurons of the NAc, their functional output has been directly linked to addiction-related behaviors (5, 37, 38), and changes in the excitatory drive onto NAc MSNs causes addiction-like behavioral abnormalities (39). Multiple proteins regulated by drugs of abuse have been linked to changes in the physiology of NAc MSNs (4, 19, 40, 41). Whereas some previous studies have examined the synaptic differences between direct and indirect pathway MSNs (2, 4) and others have focused on differences between the NAc shell and core subregions (16), a complete picture of how a given protein affects the physiology of both MSN subtypes in both NAc subregions has not previously been provided. We now show that short-term expression of ΔFosB has cell type-specific and region-specific effects on excitatory synaptic properties of NAc MSNs and that this molecular manipulation specifically in D1 NAc MSNs enhances behavioral responses to cocaine. Because the two cell types in both subregions share common proteins and signaling pathways yet have divergent effects on behavior, these results present an initial step in providing a comprehensive understanding of the molecular and circuit adaptations in the NAc caused by drugs of abuse. Future work will need to define whether these synaptic changes preferentially occur at specific inputs into the NAc, whether there is a direct causal relationship between these ΔFosB-induced synaptic and behavioral changes, and whether administration of drugs of abuse or stress, both of which induce ΔFosB, cause similar synaptic and behavioral adaptations.

Methods
For a detailed description of methods, see SI Methods.

Heterozygous bacterial artificial chromosome (BAC) adult (8–12 wk) male mice were used in all experiments and were housed together in groups of two to five per cage on a 12:12-h light/dark cycle, with food and water available at all times. Electrophysiology studies used D1-tdTomato mice backcrossed onto C57Bl/6J (21). For behavioral experiments and dextrin spiny analyses, D1- and D2-Cre mice divided into age-matched groups were used (5, 42). All experiments were done in accordance with the policies set out by institutional animal care and use committees (IACUC) at Stanford University and Mount Sinai School of Medicine.

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