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Functional Role of the N-Terminal Domain of FosB in Response to Stress and Drugs of Abuse

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Abstract

Previous work has implicated the transcription factor, FosB, acting in the nucleus accumbens, in mediating the pro-rewarding effects of drugs of abuse such as cocaine as well as in mediating resilience to chronic social stress. However, the transgenic and viral gene transfer models used to establish these FosB phenotypes express, in addition to FosB, an alternative translation product of *FosB* mRNA, termed Δ FosB, which lacks the N-terminal 78 aa present in FosB. To study the possible contribution of Δ FosB to these drug and stress phenotypes, we prepared a viral vector that overexpresses a point mutant form of *FosB* mRNA which cannot undergo alternative translation as well as a vector that overexpresses Δ FosB alone. Our results show that the mutant form of FosB, when overexpressed in the nucleus accumbens, reproduces the enhancement of reward and of resilience seen with our earlier models, with no effects seen for Δ FosB. Overexpression of full length FosB, the other major product of the *FosB* gene, also has no effect. These findings confirm the unique role of FosB in nucleus accumbens in controlling responses to drugs of abuse and stress.

INTRODUCTION

FosB is encoded by the *FosB* gene and shares homology with other Fos family transcription factors, which include c-Fos, FosB, Fra1, and Fra2. All Fos family proteins are induced rapidly and transiently in specific brain regions after acute administration of many drugs of abuse [see Nestler, 2008]. These responses are seen most prominently in nucleus accumbens (NAc) 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. In contrast, FosB, due to its unusual stability in vitro and in vivo (Ulery et al., 2006; Carle et al., 2006; Ulery-

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Reynolds et al., 2009), accumulates uniquely within the same brain regions after repeated drug exposure (Hope et al., 1994; Hiroi et al., 1997; Perrotti et al., 2008). More recent studies have demonstrated that chronic exposure to certain forms of stress also induces the accumulation of FosB in the NAc, and that such induction occurs preferentially in animals that are relatively resistant to the deleterious effects of the stress (i.e., resilient animals) (Perrotti et al., 2004; Vialou et al., 2010a, 2010b).

We have demonstrated that overexpression of FosB in the NAc, either in inducible bitransgenic mice or by local viral-mediated gene transfer, increases an animal's sensitivity to the rewarding and locomotor-activating effects of cocaine and other drugs of abuse (Kelz et al., 1999; Colby et al., 2003; Zachariou et al., 2006; Grueter et al., 2013; Robison et al., 2013). Such induction also boosts consumption of and motivation for natural rewards (Werme et al., 2002; Teegarden et al., 2007; Wallace et al., 2008; Hedges et al., 2009; Been et al., 2013; Pitchers et al., 2009; 2013), increases brain stimulation reward in intra-cranial self-stimulation paradigms (Muschamps et al., 2012), and renders animals more resilient to several forms of chronic stress (Vialou et al., 2010a, 2010b). Likewise, mice that constitutively lack expression of full length FosB, but show increased expression of $\Delta 2$ FosB, display reduced sensitivity to stress (Ohnishi et al., 2011). Together, these findings support the view that FosB, acting in the NAc, boosts an animal's state of reward, mood, and motivation.

However, a major caveat of these studies is that another product of the *FosB* gene, termed $\Delta 2$ FosB, is also expressed in all of these genetic mutant mice and viral vector systems, leaving open the possible contribution of $\Delta 2$ FosB to the behavioral phenotypes observed.

$\Delta 2$ FosB is translated from an alternative start codon located within the *FosB* mRNA transcript (Chen et al., 1997). This alternative translation leads to the formation of $\Delta 2$ FosB, which lacks the 78 N-terminal aa of FosB. In this study, we examined the role of $\Delta 2$ FosB in drug abuse and stress models by overexpressing it, or FosB or FosB, with AAV (adeno-associated virus) vectors; we used a mutant form of *FosB* mRNA that cannot undergo this alternative translation mechanism. Our results confirm that the pro-reward and pro-resilient actions seen in earlier studies are indeed mediated via FosB and not by the two other protein products of the *FosB* gene, full-length FosB or $\Delta 2$ FosB.

METHODS

Animals

Prior to experimentation, 9- to 11-week-old C57BL/6J male mice (The Jackson Laboratory, Bar Harbor, ME, USA) were group housed at five per cage in a colony room set at constant temperature (23°C) on a 12 hr light/dark cycle (lights on at 7 AM) with ad libitum access to food and water. Some experiments utilized bitransgenic mice in which overexpression of FosB is under the control of the tetracycline gene regulation system, as described (Kelz et al., 1999). Mice were used on doxycycline (to maintain gene expression off) or off doxycycline which enables FosB expression. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Mount Sinai.

AAV vectors

We used AAV2 serotype for packaging AAV vectors expressing full-length FosB, FosB, or 2 FosB under the human immediate early cytomegalovirus (CMV) promoter with Venus fluorescent protein encoded after an intervening IRES2 (internal ribosome re-entry site 2). The AAV- FosB construct expressed a mutant form of FosB mRNA, where the codon representing Met79 was mutated to Leu to obliterate the alternative translation start site that generates 2 FosB.

Viral-mediated gene transfer

Mice were positioned in small animal stereotaxic instruments under ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia, and their cranial surfaces were exposed. Thirty-three gauge syringe needles were bilaterally lowered into the NAc to infuse 0.5 μ l of AAV vector at a 10° angle (anterior/posterior + 1.6; medial/lateral + 1.5; dorsal/ventral – 4.4 mm). Infusions occurred at a rate of 0.1 μ l/min. Animals receiving AAV injections were allowed to recover for at least 24 hr after surgery. For confirmation of expression, mice were anesthetized and perfused intracardially with 4% paraformaldehyde/PBS (phosphate-buffered saline). Brains were cryoprotected with 30% sucrose, and then frozen and stored at –80°C until use. Coronal sections (40 μ m) were cut on a cryostat and processed for scanning by confocal microscopy.

Behavioral testing

Mice were studied with several standard behavioral assays according to published protocols as follows:

Chronic (10 days) *social defeat stress* was performed exactly as described (Berton et al., 2006; Vialou et al., 2010a). Briefly, one experimental mouse and one CD1 aggressor were put together for 5 min in the CD1 mouse's home cage. They were then separated by a plastic divider, which was perforated to allow for sensory contact for the remainder of the day. Every morning for 10 days, the experimental mouse was moved into a different aggressor mouse's cage. Non-defeated control mice underwent similar exposures, but with other C57BL/6J mice. Tests for *social interaction* were performed as previously described (Berton et al., 2006; Vialou et al., 2010a). Briefly, the test mouse was placed within a novel arena that included a small cage on one side. Movement (e.g., distance traveled, spent time in the vicinity of this small cage) was monitored initially for 150 sec when the small cage was empty, followed by an additional 150 sec with a CD1 mouse in that cage. Movement information was obtained using EthoVision 5.0 software (Noldus).

We used a standard, unbiased *conditioned place preference* (CPP) procedure (Kelz et al., 1999; Robison et al., 2013). Briefly, animals were pretested for 20 min in a photo-beam monitored three-chambered box with free access to environmentally distinct side-chambers. The mice were then divided into control and experimental groups with equivalent pretest scores. After experimental manipulation, mice underwent four 30 min training sessions (alternating cocaine and saline pairing). On the test day, mice had 20 min of unrestricted access to all chambers, and a CPP score was calculated by subtracting time spent in the cocaine-paired chamber minus time spent in the saline-paired chamber. Cocaine-induced

locomotor activity was measured via photobeam breaks in the CPP box for 30 min following each test injection.

Elevated plus maze tests were performed using black Plexiglass fitted with white bottom surfaces to provide contrast (Monteggia et al., 2007). Mice were placed in the center of the plus maze and allowed to freely explore the maze for 5 min under red-light conditions. The position of each mouse over time in the open and closed arms was monitored with videotracking equipment (Ethovision) and a ceiling-mounted camera.

General, ambulatory *locomotor activity* during the night phase was assessed in home cages with a photocell grid device (Med Associates Inc., St. Albans, VT, USA) that counted the number of ambulatory photo beam breaks during a 12 hr period (Roybal et al., 2007).

Western blotting

NAc samples were subjected to Western blotting as described (Vialou et al., 2010a, 2010b). Frozen NAc dissections were homogenized in 100 μ l of buffer containing phosphatase inhibitor cocktails I and II (Sigma, St. Louis, MO, USA) and protease inhibitors (Roche, Basel, Switzerland) using an ultrasonic processor (Cole Parmer, Vernon Hills, IL, USA). Protein concentrations were determined using a DC protein assay (Bio-Rad, Hercules, CA, USA), and 10–30 μ g of protein were loaded onto 12.5% or 4%–15% gradient Tris-HCl polyacrylamide gels for electrophoresis fractionation (Bio-Rad). After transferring proteins to nitrocellulose filters, the filters were incubated with an anti-FosB antibody that recognizes all *FosB* gene products, then with secondary antibody, and finally quantified using the Odyssey system (Li-Cor) according to manufacturer protocols.

Statistics

ANOVAs and student's t-tests were used, corrected for multiple comparisons, with significance set at $p < 0.05$.

RESULTS

As shown in Figure 1A, the *FosB* gene encodes mRNAs for full-length FosB and for FosB.

FosB mRNA is generated from an alternative splicing event within Exon 4 of the *FosB* primary transcript; this results in the generation of a premature stop codon and to the truncated FosB protein, which lacks the C-terminal 101 aa present in FosB. FosB and

FosB mRNA share the same ATG start codon, located toward the 3' end of Exon 1. It has been known since the original cloning of *FosB* products that the two mRNAs also share alternative translation start sites within Exon 2, termed the 1, 2, and 3 ATGs. Previous work showed that a minor protein product is generated from *FosB* mRNA, but not *FosB* mRNA, via the 2 ATG; this protein is termed 2 FosB and lacks the 78 aa N-terminal region of FosB (Chen et al., 1997). In contrast, the 1 and 3 ATGs appear to be silent, since there is no evidence for their use in translation of the *FosB* or *FosB* transcripts.

Figure 1B illustrates the induction of *FosB* gene products in NAc after a course of repeated cocaine administration, with animals examined 2 hr after the last cocaine dose. At this time point, both FosB and FosB proteins show significant induction by cocaine, with no

consistent induction of Δ FosB. Note that the induction of both FosB and Δ FosB is different from the pattern seen at 24 hr or more after the last drug dose, when only FosB is induced due to the unique stability of the FosB protein (Hope et al., 1994; Chen et al., 1997; Perrotti et al., 2008). However, in contrast to the lack of induction of Δ FosB by repeated cocaine administration, the bitransgenic mouse system that we have used to overexpress FosB and to thereby study its behavioral consequences (Kelz et al., 1999; Colby et al., 2003; Zachariou et al., 2006) leads to a significant, albeit lower levels of, overexpression of Δ FosB in addition to FosB (Figure 1C). A similar level of induction of Δ FosB is seen with our viral vectors that overexpress wildtype *FosB* (e.g., see Figure 2). These observations raise the possibility that some of the purported actions of FosB reported previously might be mediated in part via Δ FosB.

To distinguish the differential roles of FosB versus Δ FosB, we generated an AAV vector that overexpresses Δ FosB alone, as well as a new vector that overexpresses a mutant form of *FosB* mRNA (*m FosB* mRNA) which cannot be subject to alternative translation to generate Δ FosB. Both vectors also express Venus as a marker of expression. We compared the effects of these two vectors to others, which express FosB plus Venus or Venus alone as a control. The ability of these new AAV vectors to selectively overexpress their encoded transgenes is depicted in Figure 2.

Next, to test the effect of each *FosB* gene product, acting in the NAc, on complex behavior, we injected each of these AAVs into this brain region bilaterally of separate groups of mice and, 3 weeks later when transgene expression is maximal (Figure 3A), performed a battery of tests. We first evaluated the ability of the *FosB* gene products to influence the pro-resilience phenotype reported previously for FosB in the social defeat paradigm (Vialou et al., 2010a, 2010b). As shown in Figure 3A, control mice expressing Venus alone displayed the expected decrement in social interaction behavior, a well-established behavioral marker of susceptibility (Berton et al., 2006; Vialou et al., 2010a). Overexpression of *m FosB* completely reversed this phenotype, in contrast to Δ FosB and FosB which had no effect.

To test the relative contribution of each *FosB* gene product to the rewarding effects of cocaine, we overexpressed Δ FosB itself, *m FosB*, or FosB bilaterally in the NAc and studied the animals in the conditioned place preference paradigm. As shown in Figure 3B, bilateral overexpression of *m FosB* in the NAc increases the place conditioning effects of a threshold dose of cocaine, which did not produce a significant place preference in Venus-expressing control animals. In contrast, overexpression of Δ FosB or FosB had no effect on cocaine place conditioning. Since we used a threshold dose of cocaine, which did not produce a significant place preference in control animals, we cannot exclude the possibility that FosB or Δ FosB might reduce the rewarding effects of cocaine.

Finally, to evaluate baseline behaviors, we examined locomotor activity in the animals' home cage as well as anxiety-like behavior in the elevated plus maze. FosB, *m FosB*, nor Δ FosB overexpression in the NAc had an effect on locomotor activity, although FosB and Δ FosB—but not *m FosB*—produced a small but significant decrease in anxiety-like behavior in the elevated plus maze (Figure 3D,E). These data suggest that *FosB* gene expression does not appreciably alter behavior under normal conditions.

DISCUSSION

Results of the present study confirm that the phenotype reported previously for FosB is indeed mediated via FosB and not by Δ FosB, an alternatively translated product of *FosB* mRNA which lacks FosB's N-terminus. While our previously used tools to overexpress FosB also result in the generation of low levels of Δ FosB, we show here that overexpression in NAc of a mutated form of *FosB* mRNA, which cannot generate Δ FosB due to mutation of the alternative start codon involved, recapitulates the increase both in cocaine reward and in resilience to social defeat stress reported previously for FosB (Kelz et al., 1999; Vialou et al., 2010a). Moreover, overexpression of Δ FosB itself has no effect on either cocaine or stress responses. We also show, for the first time, that overexpression of full length FosB in NAc likewise has no effect on behavioral responses to cocaine or stress.

While these results do not rule out the possibility that Δ FosB, as a minor protein product of the *FosB* gene, might exert functional effects in other brain regions or in peripheral tissues, our findings nonetheless confirm the unique contribution of FosB, acting in the NAc reward circuit, in promoting cocaine reward and stress resilience.

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References

- Been LE, Hedges VL, Vialou V, Nestler EJ, Meisel RL. Delta JunD expression in the nucleus accumbens prevents sexual reward in female Syrian hamsters. *Genes Brain Behav.* 2013; 12:666–672. [PubMed: 23790091]
- Berton O, McClung CA, DiLeone RJ, Krishnan V, Russo S, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science.* 2006; 311:864–868. [PubMed: 16469931]
- Carle TL, Ohnishi YN, Ohnishi YH, Alibhai IN, Wilkinson MB, Kumar A, Nestler EJ. Absence of conserved C-terminal degron domain contributes to FosB's unique stability. *Eur J Neurosci.* 2007; 25:3009–3019. [PubMed: 17561814]
- Chen JS, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ. Chronic Fos-related antigens: stable variants of deltaFosB induced in brain by chronic treatments. *J Neurosci.* 1997; 17:4933–4941. [PubMed: 9185531]
- Colby CR, Whisler K, Steffen C, Nestler EJ, Self DW. FosB enhances incentive for cocaine. *J Neurosci.* 2003; 23:2488–2493. [PubMed: 12657709]
- Grueter BA, Robison AJ, Neve RL, Nestler EJ, Malenka RC. FosB differentially modulates nucleus accumbens direct and indirect pathway function. *Proc Natl Acad Sci USA.* 2013; 110:1923–1927. [PubMed: 23319622]
- Hedges VL, Chakravarty S, Nestler EJ, Meisel RL. FosB overexpression in the nucleus accumbens enhances sexual reward in female Syrian hamsters. *Genes Brain Behav.* 2009; 8:442–449. [PubMed: 19566711]
- Hiroi N, Brown J, Haile C, Ye H, Greenberg ME, Nestler EJ. 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.* 1997; 94:10397–10402. [PubMed: 9294222]

- Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS, Nestler EJ. Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron*. 1994; 13:1235–1244. [PubMed: 7946359]
- Kelz MB, Chen J, Carlezon WA Jr, Whisler K, Gilden L, Beckmann AM, Steffen C, Zhang Y-J, Marotti L, Self DW, Tkatch R, Baranaukas G, Surmeier DJ, Neve RL, Duman RS, Picciotto MR, Nestler EJ. Expression of the transcription factor FosB in the brain controls sensitivity to cocaine. *Nature*. 1999; 401:272–276. [PubMed: 10499584]
- Monteggia LM, Luikart B, Barrot M, Theobald D, Malkovska I, Nef S, Parada LF, Nestler EJ. BDNF conditional knockouts show gender differences in depression related behaviors. *Biol Psychiatry*. 2007; 61:187–197. [PubMed: 16697351]
- Muschamp JW, Nemeth CL, Robison AJ, Nestler EJ, Carlezon WA Jr. FosB enhances the rewarding effects of cocaine while reducing the pro-depressive effects of the kappa-opioid agonist U50488. *Biol Psychiatry*. 2012; 71:44–50. [PubMed: 21962331]
- Nestler EJ. Transcriptional mechanisms of addiction: role of deltaFosB. *Philos Trans R Soc London B Biol Sci*. 2008; 363:3245–3255. [PubMed: 18640924]
- Ohnishi YN, Ohnishi YH, Hokama M, Nomaru H, Yamazaki K, Tominaga Y, Sakumi K, Nestler EJ, Nakabeppu Y. FosB is essential for the enhancement of stress tolerance and antagonizes locomotor sensitization by FosB. *Biol Psychiatry*. 2011; 70:487–495. [PubMed: 21679928]
- Perrotti LI, Hadeishi Y, Ulery P, Barrot M, Monteggia L, Duman RS, Nestler EJ. Induction of FosB in reward-related brain regions after chronic stress. *J Neurosci*. 2004; 24:10594–10602. [PubMed: 15564575]
- Perrotti LI, Weaver RR, Robison B, Renthal W, Maze I, Yazdani S, Elmore RG, Knapp DJ, Selley DE, Martin BR, Sim-Selley L, Bachtell RK, Self DW, Nestler EJ. Distinct patterns of FosB induction in brain by drugs of abuse. *Synapse*. 2008; 62:358–369. [PubMed: 18293355]
- Pitchers KK, Frohmader KS, Vialou V, Mouzon E, Nestler EJ, Lehman MN, Coolen LM. FosB in the nucleus accumbens is critical for reinforcing effects of sexual reward. *Genes Brain Behav*. 2010; 9:831–840. [PubMed: 20618447]
- Pitchers KK, Vialou V, Nestler EJ, Lehman MN, Coolen LM. Sexual experience increases amphetamine reward and nucleus accumbens spinogenesis via dopamine D1 receptor activity and induction of deltaFosB. *J Neurosci*. 2013; 33:3434–3442. [PubMed: 23426671]
- Roybal K, Theobald D, DiNieri JA, Graham A, Russo S, Krishnan V, Chakravarty S, Peevey J, Oehrlein N, Birnbaum S, Vitaterna MH, Orsulak P, Takahashi JS, Nestler EJ, Carlezon WA Jr, McClung CA. Mania-like behavior induced by disruption of CLOCK. *Proc Natl Acad Sci USA*. 2007; 104:6406–6411. [PubMed: 17379666]
- Teegarden SL, Bale TL. Decreases in dietary preference produce increased emotionality and risk for dietary relapse. *Biol Psychiatry*. 2007; 61:1021–1029. [PubMed: 17207778]
- Ulery PG, Rudenko G, Nestler EJ. Regulation of FosB stability by phosphorylation. *J Neurosci*. 2006; 26:5131–5142. [PubMed: 16687504]
- Ulery-Reynolds PG, Castillo MA, Vialou V, Russo SJ, Nestler EJ. Phosphorylation of FosB mediates its stability in vivo. *Neuroscience*. 2009; 158:369–372. [PubMed: 19041372]
- Vialou V, Robison AJ, LaPlant QC, Covington HE III, Dietz DM, Ohnishi YN, Mouzon E, Rush AJ III, Watts EL, Wallace DL, Iniguez SD, Ohnishi YH, Steiner MA, Warren B, Krishnan V, Neve RL, Ghose S, Berton O, Tamminga CA, Nestler EJ. FosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nature Neurosci*. 2010a; 13:745–752. [PubMed: 20473292]
- Vialou V, Maze I, Renthal W, LaPlant QC, Watts EL, Mouzon E, Ghose S, Tamminga CA, Nestler EJ. Serum response factor promotes resilience to chronic social stress through the induction of FosB. *J Neurosci*. 2010b; 30:14585–14592. [PubMed: 20980616]
- Wallace DL, Vialou V, Rios L, Carle-Florence TL, Chakravarty S, Kumar A, Graham D, Green TA, Iniguez SD, Perrotti LI, Barrot M, DiLeone RJ, Nestler EJ, Bolaños CA. The influence of FosB in the nucleus accumbens on natural reward-related behavior. *J Neurosci*. 2008; 28:10272–10277. [PubMed: 18842886]
- Werme M, Messer C, Olson L, Gilden L, Thorén P, Nestler EJ, Brené S. FosB regulates wheel running. *J Neurosci*. 2002; 22:8133–8138. [PubMed: 12223567]

Zachariou V, Bolanos CA, Selley DE, Theobald D, Cassidy MP, Kelz MB, Shaw-Lutchman T, Berton O, Sim-Selley LJ, DiLeone RJ, Kumar A, Nestler EJ. FosB: An essential role for FosB in the nucleus accumbens in morphine action. *Nature Neurosci.* 2006; 9:205–211. [PubMed: 16415864]

Highlights

- *FosB* mRNA gives rise to FosB and to the minor alternatively translated 2 FosB.
- Overexpression of FosB alone confirms its pro-reward and pro-resilience phenotype.
- In contrast, 2 FosB has no effect on cocaine reward or stress vulnerability.
- Full-length FosB, encoded by *FosB* mRNA, also does not affect reward or resilience.

Figure 1. Expression levels of *FosB* gene products

A) Scheme showing that the *FosB* gene produces two mRNAs, *FosB* and *FosB*. *FosB* mRNA arises from alternative splicing within Exon 4 of the *FosB* primary transcript; this splicing generates a premature stop codon and the truncated *FosB* protein. *FosB* is generated from an alternative start codon corresponding to aa 79 in *FosB*. The 1 and 3 ATGs, also present in the *FosB* mRNA sequence, are not used as alternative start sites (Chen et al., 1997). The position of the BZIP domain present in all *FosB* gene products is shown. B) Western blotting of *FosB* gene products in NAc after repeated administration of cocaine (C, 15 mg/kg i.p., n=4-5) or saline (S) for 7 days, with mice analyzed 2 hr after the last injection. C) Western blotting of *FosB* gene products in NAc in bitransgenic mice that

overexpress FosB (Kelz et al., 1999), and to a much lesser extent 2 FosB, in the absence of doxycycline (DOX) (n=3–6). Histone H3 or GAPDH (glyceraldehyde 3-phosphate dehydrogenase), which are not regulated under the experimental conditions used, were utilized as a loading control. *p<0.05 by t-tests.

Figure 2. Selective expression of *FosB* gene products with AAV vectors in Neuro2A cells
 Neuro2A cells were transfected with 0.25 μ g of each AAV vector, or mock transfected with no vector, and cell extracts were analyzed by Western blotting. Arrowhead denotes full length FosB, two black arrows FosB, and open arrow 2 FosB. Note the much lower levels of 2 FosB expressed by wildtype *FosB* mRNA but not by *m FosB* mRNA which is not subject to alternative translation.

Figure 3. Effect of *FosB* gene products in NAc on behavioral responses to cocaine or social stress
AAV vectors described in Figure 2 were injected bilaterally into the NAc and separate groups of animals were studied in a battery of behavioral assays. A) Shows the targeting of the medial NAc by the viral vectors. AC, anterior commissure. B) Shows results of social interaction test one day after 10 days of social defeat stress (n=22–40). **p < 0.01, ***p < 0.001 by t-tests. C) Shows conditioned place preference for a threshold dose of cocaine (7.5 mg/kg i.p.) (F(3,107)=4.890, p < 0.001). *p < 0.05, **p < 0.01, ***p < 0.001 in post-hoc test after ANOVA. D) Percent time spent in the open arms of an elevated plus maze. FosB and Δ2 FosB increased time in the open arms compared to Venus controls

(* $p < 0.05$ by t-tests), an effect not seen for FosB (n=22–40). E) General locomotor activity in the animals' home cage during the dark phase. No *FosB* gene product was different from Venus controls (n=22–40).