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Striatal regulation of ΔFosB, FosB, and cFos during cocaine self-administration and withdrawal

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Abstract

Chronic drug exposure induces alterations in gene expression profiles that are thought to underlie the development of drug addiction. The present study examined regulation of the Fos-family of transcription factors, specifically cFos, FosB, and ΔFosB, in striatal subregions during and after chronic intravenous cocaine administration in self-administering and yoked rats. We found that cFos, FosB, and ΔFosB exhibit regionally and temporally distinct expression patterns, with greater accumulation of ΔFosB protein in the nucleus accumbens (NAc) shell and core after chronic cocaine administration, while ΔFosB increases in the caudate-putamen (CPu) remained similar with either acute or chronic administration. In contrast, tolerance developed to cocaine-induced mRNA for ΔFosB in all 3 striatal subregions with chronic administration. Tolerance also developed to FosB expression, most notably in the NAc shell and CPu. Interestingly, tolerance to cocaine-induced cFos induction was dependent on volitional control of cocaine intake in ventral but not dorsal striatal regions, whereas regulation of FosB and ΔFosB was similar in cocaine self-administering and yoked animals. Thus, ΔFosB-mediated neuroadaptations in the CPu may occur earlier than previously thought with the initiation of intravenous cocaine use and, together with greater accumulation of ΔFosB in the NAc, could contribute to addiction-related increases in cocaine-seeking behavior.

Keywords

cocaine; self-administration; withdrawal; striatum; Fos

Introduction

Repeated exposure to addictive drugs produces neuroadaptations in brain reward pathways that are thought to underlie both the development of compulsive drug-taking and the persistence of craving and relapse to drug-seeking behavior in withdrawal. Many of these neuroadaptations result from the induction of transcription factors and subsequent regulation of gene expression, which can have potentially long-lasting effects on neuronal structure and function (Zhang *et al.* 2006). The Fos family of transcription factors is of particular interest, as members of this family show differential induction patterns in striatal regions after both acute and chronic cocaine exposure. When cocaine is administered acutely in a passive, non-

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contingent fashion (i.e., by intraperitoneal (IP) injection) it increases cFos and FosB mRNA and protein in both the dorsal (caudate-putamen, CPu) and ventral (nucleus accumbens, NAc) striatum (Graybiel *et al.* 1990; Young *et al.* 1991; Hope *et al.* 1992), while tolerance to this response occurs with chronic passive administration (Hope *et al.* 1992, 1994; Alibhai *et al.* 2007). In contrast, striatal levels of ΔFosB (35-37 kDa), a stable truncated splice variant of the *fosB* gene, are elevated after chronic but not acute passive cocaine exposure (Hope *et al.* 1994; Nye *et al.* 1995; Chen *et al.* 1995, 1997). These stable ΔFosB isoforms can heterodimerize with different Jun family proteins than either cFos or FosB (Chen *et al.* 1995), and can also form functional homodimers with itself (Jorissen *et al.* 1997), suggesting that the differential formation of activator protein-1 (AP-1) complexes after chronic cocaine may alter gene expression at AP-1 sites in a way that is distinct from gene expression produced by acute cocaine exposure (Hope, 1998; Kelz and Nestler, 2000). Differential changes in gene expression profiles also occur depending on whether ΔFosB elevations are short-term or long-lasting, and these changes may lead to differential expression of cocaine-mediated behaviors (McClung and Nestler, 2003). Chronic exposure to other drugs including amphetamine, morphine, Δ^9-THC, nicotine, ethanol, and phencyclidine also leads to accumulation of stable ΔFosB isoforms in striatal regions (McClung *et al.* 2004; Perrotti *et al.* 2008). Furthermore, recent findings suggest a negative interaction between ΔFosB accumulation and amphetamine-induced cFos that may account for tolerance to cFos induction found after chronic stimulant exposure (Renthal *et al.* 2008). Together, these findings have led to the hypothesis that stable ΔFosB isoforms may act as a "molecular switch" and facilitate the transition from initial drug use to more addicted biological states (Nestler *et al.* 2001; Nestler, 2008).

While most previous studies utilized repeated passive cocaine treatments to study expression of Fos family proteins, and there are relatively few examples of this regulation when cocaine is self-administered intravenously (IV) for several hours typical of human abuse patterns. One study found that cFos mRNA is elevated in the CPu after a single 30-min session of cocaine self-administration in mice (Kuzmin and Johansson, 1999), while no changes have been found in the CPu of rats after either sub-chronic (3 days) or chronic (6-12 weeks) cocaine self-administration (Daunais *et al.* 1993, 1995). After a period of withdrawal, cocaine-mediated increases in cFos protein in the NAc is reduced in rats with prior escalated cocaine intake (Ben-Shahar *et al.* 2004), while elevated cFos levels are found throughout the striatum after exposure to cocaine-associated cues (Neisewander *et al.* 2000; Kufahl *et al.* 2009). In contrast to cFos, increased protein levels of ΔFosB have been shown throughout the striatum after chronic cocaine self-administration, and this accumulation can persist for at least 1 day into withdrawal (Pich *et al.* 1997; Perotti *et al.* 2008). However, there are no reports that compare changes in the responsiveness of multiple Fos family proteins to such intravenous cocaine administration with either acute or chronic exposure. Given the potential interactions between ΔFosB and cFos, the ability of differential AP-1 complex formation to produce differential effects on gene expression, and the possible impact of these differences on cocaine-mediated behavior, it is also important to confirm that the alterations in the expression of cFos, FosB, and ΔFosB that occur after non-contingent administration also are found when cocaine is self-administered voluntarily, and to determine how long these alterations may persist after cocaine administration is terminated. Therefore, in the present study we compared the effects of chronic IV cocaine administration on expression of ΔFosB, FosB, and cFos in striatal subregions during both cocaine administration and withdrawal. We compared regulation found with volitional self-administration with regulation in animals receiving an identical amount and temporal pattern of cocaine through non-volitional yoked infusions after either acute or chronic exposure. Given that FosB and ΔFosB are splice variants of the same *fosB* gene, we also compared regulation of mRNAs for FosB and ΔFosB with regulation at the protein level.

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Experimental Procedures

Subjects and Surgery

Adult male Sprague-Dawley rats initially weighing approximately 250-300 g were housed in temperature- and humidity-controlled environment on a 12 h light-dark cycle (lights on at 7:00 AM). Animals were fed food and water *ad libitum* at all times with the exception that they were maintained at 85% of their free feeding weight during lever-press training for sucrose pellets (45 mg, BioServ). Lever-press training was conducted in ventilated operant chambers (Med Associates, Georgia, VT) until acquisition criteria were met (100 pellets per session for 3 consecutive sessions) under a fixed-ratio 1 (FR1) reinforcement schedule. Animals were then fed *ad libitum* for at least 24 h prior to surgery. For surgery, rats were given atropine (0.04 mg/kg, subcutaneous) to aide respiration and a chronic, indwelling catheter was inserted into the right jugular vein under sodium pentobarbital (50 mg/kg, IP) anesthesia according to previously published procedures (Edwards *et al.* 2007a). After surgery, rats were given an injection of penicillin (200,000 IU/kg, intramuscular) to prevent infection, and catheters were flushed daily with 0.2 ml heparinized (20 IU/ml) bacteriostatic saline containing gentamycin sulphate (0.33 mg/ml). All experimental procedures were conducted in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*, and were approved by the UT Southwestern Medical Center Institutional Animal Care and Use Committee (IACUC).

Apparatus and Self-administration procedures

Following 1 wk recovery from surgery, animals were divided into multiple experimental groups/withdrawal time (Fig. 1A), and returned to the operant test chambers in daily sessions as previously described (Edwards *et al.* 2007b). Rats in the untreated control group were single housed and handled daily in their home cages without exposure to the self-administration environment. Rats in the cocaine self-administration (CSA) group were allowed to voluntarily self-administer cocaine (0.5 mg/kg/50 μl infusion) under a fixed-ratio 1 (FR1) schedule of reinforcement in daily 4 h sessions, conducted 6 days/wk, for a total of 18 days. Each active lever press produced a 2.5 s cocaine infusion that was associated with the illumination of a cue light above the active lever. The house light was extinguished during cocaine infusions, and there was an additional 12.5 s timeout period after the infusion in which the house light remained off. Lever responding during the infusion and timeout period was recorded, but had no consequence. An additional inactive lever was present in the chambers, but responding on this lever was without consequence. Rats in the chronic yoke (CY) group were paired to actively self-administering rats and received passive cocaine infusions in amounts and temporal patterns identical to their self-administering partners. Rats in the acute yoke (AY) group were also paired to rats in the chronic CSA group, but received passive saline infusions instead of cocaine until the last day of self-administration, when they received a single session of passive cocaine infusions for the first time. Finally, the Saline SA group was allowed to self-administer saline throughout to identify potential changes related to surgery, testing or other experimental procedures when compared to untreated controls. Comparisons between AY and CY groups were used to identify changes in the responsiveness of cFos, FosB, or ΔFosB with acute and chronic cocaine exposure, while CSA and CY groups were compared in order to identify changes in cFos, FosB, or ΔFosB expression that were specifically related to the rewarding versus pharmacological effects of cocaine. Tissue from all study groups was collected immediately after the final 4 h test session to compare cocaine-induced regulation of cFos, FosB, and ΔFosB, and the persistence of cocaine-induced changes was determined for some study groups with tissue collected 24 h or 3 wk after the final test session. Quantitative western blot and RT-PCR procedures were used on dissections of striatal subregions to circumvent

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potential problems relating to antibody cross-reactivity and improve sensitivity for detecting changes.

Tissue Collection

Rats were sacrificed by microwave irradiation aimed at the head region (5 kW, 1.5 s, Murimachi Kikai, Tokyo, Japan). Brains were quickly dissected and chilled, and bilateral tissue punches (14 gauge) of the nucleus accumbens (NAc) shell, NAc core, and caudate-putamen (CPu) were obtained from 1.5 mm coronal slices based on coordinates obtained from Paxinos and Watson (1998, illustrated in Figure 1B). Tissue samples were homogenized by sonication in lysis buffer containing protease and phosphatase inhibitors. Homogenates were then boiled for 5 min, placed on ice, and subsequently assayed by Lowry to determine protein concentrations. Homogenates were then aliquotted in 20 μg samples and stored at -80°C until use.

Western Blots

Tissue samples were loaded onto 12% polyacrylamide gels for separation by electrophoresis in Tris/Glycine/sodium dodecyl sulfate buffered saline solution (TGS; Bio-Rad, Hercules, CA). After separation, samples were transferred by electrophoresis (250 mA for 18 h) to polyvinylidene fluoride membranes (PVDF; Amersham, Piscataway, NJ) and subsequently blocked in 3% nonfat dry milk and 1× Tris/Tween buffered saline solution (TTBS; Bio-Rad, Hercules, CA) overnight at 4°C. Membranes were then incubated in 1:1000 dilution of primary Fra antibody (kindly provided by Dr. Michael Iadarola, National Institutes of Health, Bethesda, MD) in a 3% milk/1×TTBS solution overnight at 4° C. Membranes were washed in 1×TTBS (4 times, 15 min each), and incubated in 1×TTBS containing a 1:25000 dilution of goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (Bio-Rad, Hercules, CA) for 1 h at room temperature. Membranes were washed again and then developed using Pierce Super Signal West Dura (Thermo Fisher Scientific Inc., Rockford, IL) chemiluminescent-mediated detection on Hyperfilm (ECL plus; Amersham). The localization of cFos, FosB, and ΔFosB protein bands is illustrated in Figure 1C. We chose to examine only the stably expressed forms of ΔFosB (i.e., 35-37 kDa) in this study, as it is these forms that are thought to accumulate with chronic drug use and produce the neuroplasticity that underlies addiction (Nestler *et al.* 2001). Scion Image (Frederick, MD) was used to assign absolute immunoreactivity to the bands, and a scanner was used to take digital images of the films. After detection, the membranes were stripped and re-probed for β-tubulin (1:200000, Cell Signaling, Danvers, MA). β-tubulin levels were used as a loading control to normalize levels of Fos-related proteins.

RT-PCR

Quantitative RT-PCR (qRT-PCR) was used to determine changes of FosB and ΔFosB mRNA immediately and 24 h following cocaine administration. Animals were euthanized by rapid decapitation and NAc core, NAc shell, and CPu were isolated as described (Graham *et al.* 2007; Bachtell *et al.* 2008). Individual samples were immediately homogenized in RNA-STAT-60 (IsoTex Diagnostics Inc, Friendswood, TX) and frozen on dry ice until mRNA was extracted according to the manufacturer's instructions. Briefly, chloroform was added to each sample and the aqueous layer was isolated following centrifugation. Total mRNA was precipitated with isopropanol in the presence of linear acrylamide (Ambion, Austin, TX). Samples were centrifuged and the extracted mRNA pellets were washed with 70% ethanol and resuspended in DEPC water. Total mRNA was DNAase treated (Ambion, Foster City, CA) and reverse-transcribed to cDNA with random hexamers using Superscript III (Invitrogen, Carlsbad, CA). Primer sequences used to amplify FosB, ΔFosB, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5′-GTGAGAGATTTGCCAGGGTC-3′ and 5′-AGAGAGAAGCCGTCAGGTTG-3′, 5′-

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AGGCAGAGCTGGAGTCGGAGAT-3′ and 5′-GCCGAGGACTTGAACTTCACTCG-3′, and 5′-AGGTCGGTGTGAACGGATTTG-3′ and 5′-TGTAGACCATGTAGTTGAGGTCA-3′, respectively. Cycle thresholds (cT) were calculated from triplicate reactions using the second derivative of the amplification curve. FosB and ΔFosB cT values were normalized to GAPDH cT values (ΔcT) since GAPDH was not regulated by cocaine. Fold changes were calculated using the ΔΔcT method as described previously (Applied Biosystems manual).

Statistical analysis

The levels of each protein were expressed as % change from untreated controls for each brain region and time point, and study groups were compared by one-way analysis of variance (ANOVA), with the significance level set at $p < 0.05$. Overall effects were followed by post-hoc comparisons using Fishers LSD tests. Correlations between cocaine intake and changes in protein levels were assessed using linear regression.

Results

Animals in the CSA group that were allowed to voluntarily self-administer cocaine exhibited stable patterns of cocaine self-administration by the third week of SA (days 13-18). Over the final week of SA the average daily cocaine intake in CSA rats and their CY partners was 46.9 (±1.8) mg/kg/day (range: 37-60 mg/kg/day). On the final test day, CSA rats in the 0 h withdrawal (WD) group self-administered 44.5 (±2.5) mg/kg cocaine (range 25.5-57.5 mg/kg), and an identical amount of cocaine was received by their CY and AY partners.

Differential regulation of ΔFosB protein in striatal subregions after acute or chronic cocaine

Differential regulation of ΔFosB protein was found in striatal subregions immediately following 4 h of intravenous cocaine administration (0 h WD). In the NAc shell, only chronic cocaine produced significant increases in CSA and CY groups (45-61%) compared to untreated controls (Fig. 2A, $F_{4,60} = 4.22$, $p = 0.005$). In the NAc core, significant increases in ΔFosB (41%) were found after acute exposure in the AY group (Fig. 2B, $F_{4,60} = 17.04$, $p < 0.001$), and even larger increases (89-95%) were found after chronic cocaine. In contrast to greater accumulation of ΔFosB in the NAc with chronic cocaine administration, the CPu showed similar increases in ΔFosB (86-102%) across both acute and chronic cocaine groups (Fig. 2C, $F_{4,78} = 19.09$, $p < 0.001$). There was no difference in ΔFosB increases between CSA and CY groups in any striatal subregion, indicating that regulation was related to cocaine exposure irrespective of volitional cocaine consumption. Regulation of ΔFosB persisted for at least 24 h after chronic cocaine in the NAc shell ($F_{2,32} = 5.19$, $p = 0.02$), NAc core ($F_{4,60} = 4.53$, $p = 0.02$), and CPu ($F_{2,34} = 12.13$, $p < 0.001$), but returned to baseline levels after 3 wk. Similar increases in ΔFosB were found when cocaine groups were compared to the Saline SA group, except that smaller increases in the NAc shell of AY animals achieved significance when compared to Saline SA, but not to untreated controls. However, there was no significant regulation of ΔFosB in animals that self-administered saline throughout training when compared to untreated controls, indicating that regulation of ΔFosB was due to cocaine and not a result of the surgical or testing procedures.

Tolerance to regulation of FosB protein after chronic cocaine

In contrast to regulation of ΔFosB, a single exposure to 4 h of IV cocaine administration produced substantially larger increases in FosB protein in all 3 striatal subregions, but substantial tolerance in this response developed after chronic cocaine administration. In the NAc shell, FosB increased (260%) immediately after 4 h of acute cocaine administration in

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AY animals, but these increases were reduced (to 142-146%) after chronic administration in both CY and CSA groups (Fig. 3A, $F_{4,77} = 23.16$, $p < 0.001$). Similar increases in FosB (295%) were found in the CPu of AY animals that also were reduced (to 135-159%) after chronic cocaine administration in CY and CSA groups (Fig. 3C, $F_{4,69} = 13.362$, $p < 0.001$). In the NAc core, acute cocaine administration produced less substantial increases in FosB (164%) in AY animals compared to the other brain regions; however, these increases were still greater than those produced after chronic administration (109-112-%) in CY and CSA groups (Fig. 3B, $F_{4,57} = 20.23$, $p < 0.001$). As found with ΔFosB, regulation of FosB after chronic cocaine was not modulated by volitional control of cocaine intake. However, in contrast to ΔFosB, increases in FosB protein failed to persist in both the NAc shell and core after 24 h, although residual increases (38-52%) persisted in the CPu ($F_{2,32} = 3.590$, $p < 0.05$). FosB levels were not affected by surgical or testing procedures in saline self-administering animals.

Attenuation of both ΔFosB and FosB mRNA induction after chronic cocaine

Acute exposure to 4 h of intravenous cocaine administration produced similar increases (11-16 fold) in ΔFosB mRNA in the NAc shell ($F_{3,19} = 15.82$, $p < 0.001$), NAc core ($F_{3,19} = 13.275$, $p < 0.001$, and CPu ($F_{3,11} = 5.78$, $p = 0.03$) when compared to Saline SA controls (0 h WD, Fig. 4A). However, this response was strongly suppressed in CY and CSA groups after chronic cocaine administration in the NAc shell (3-4 fold), NAc core (4 fold), and CPu (3 fold). Despite the fact that acute IV cocaine administration produced larger increases in FosB protein relative to ΔFosB, acute cocaine administration induced relatively lower increases in mRNA for FosB (4-9 fold) than for ΔFosB (11-16 fold) in all 3 striatal subregions (Fig. 4B). This response was virtually abolished after chronic cocaine in the NAc shell ($F_{3,19} = 26.22$, $p < 0.001$) and CPu ($F_{3,11} = 4.24$, $p < 0.05$), although small but significant increases (2 fold) remained in CY and CSA groups in the NAc core ($F_{3,19} = 11.10$, $p < 0.001$). The cocaine-induced increases in both ΔFosB and FosB in AY animals were not retained after 24 h WD when compared to this same Saline SA control group. Further analysis of the ratio of FosB to ΔFosB mRNA levels at the 0 h WD time point showed that cocaine administration markedly reduced the relative amount of FosB to ΔFosB mRNA in the NAc shell ($F_{3,19} = 4.79$, $p = 0.02$), NAc core ($F_{3,19} = 4.49$, $p = 0.02$), and CPu ($F_{3,11} = 5.59$, $p = 0.03$) due to greater formation of the ΔFosB isoform, and regardless of substantial tolerance to the cocaine-induced response in both mRNAs after chronic administration (Fig. 4C). There was no significant difference in these ratios whether cocaine was self-administered or received passively by yoked infusion, and the relative ratios of FosB:ΔFosB had returned to normal in all three brain regions by the 24h WD time point (data not shown).

Reinforcement-related tolerance to cocaine-induced cFos in the NAc

In contrast to regulation of FosB gene products that represented a pharmacological response to cocaine irrespective of passive or volitional administration, regulation of cFos in NAc subregions was strongly influenced by the context of cocaine self-administration when compared to animals receiving cocaine by passive yoked infusions. Cocaine exposure increased cFos protein levels (109-126%) both in the NAc shell and core with either acute or chronic administration in AY and CY groups (Fig. 5A-B). However, when cocaine infusions were delivered in a response-contingent manner in self-administering animals, this response was reduced (to 55%) in the NAc shell ($F_{4,60} = 9.14$, $p < 0.001$), and failed to significantly increase cFos in the NAc core ($F_{4,57} = 5.92$, $p < 0.001$). In the CPu, tolerance to cocaine-induced cFos developed with either chronic passive or volitional cocaine administration (Fig. 5C), and cFos induction in AY animals (164%) was reduced (to 45-57%) in both CY and CSA groups ($F_{4,67} = 13.29$, $p < 0.001$), similar to the development of tolerance in the induction of FosB protein in all 3 striatal subregions. Thus, reinforcement-related tolerance

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to cocaine-induced cFos occurred specifically in mesolimbic regions of the striatum. In all 3 striatal regions, increases in cFos were not found in saline self-administering animals and failed to persist after 24 h WD.

Relationship between cocaine intake, cFos and ΔFosB in striatum subregions

Since amounts of cocaine self-administration varied across individual animals and their yoked partners, we compared the amount of cocaine intake with the induction of cFos, FosB, and ΔFosB protein levels by multiple linear regression analyses (see Supplemental Table 1 for the results of all potential correlations). There were significant correlations between cocaine intake and cFos levels in rats that received acute cocaine administration by passive infusions, and these relationships differed in dorsal and ventral striatal subregions. In the NAc core, induction of cFos immediately after 4 h of acute IV cocaine administration was strongly and negatively correlated with cocaine intake, while a similar but insignificant relationship was found in the NAc shell (Fig. 6). In contrast, induction of cFos was positively correlated with cocaine intake in the CPu. There were no significant correlations between cocaine intake (either active or passive) and protein levels of FosB or ΔFosB in any striatal subregion. However, there was a strong positive correlation between levels of cFos and ΔFosB in the NAc shell 24 h after cocaine, but only in animals that received cocaine through volitional self-administration (Fig. 7), and despite the fact that overall cFos levels were not altered at 24 h WD. Similar trends ($p < 0.07$) for positive correlations between cFos and ΔFosB proteins levels were found immediately after 4 h of cocaine self-administration in the NAc core, and in the CPu of animals receiving cocaine for the first time (AY group).

Discussion

In the present study, we examined the effects of acute and chronic intravenous cocaine exposure or chronic self-administration on regulation of ΔFosB, FosB, and cFos levels in the NAc shell, NAc core, and CPu striatal subregions. Previous studies have consistently found that ΔFosB is increased only after repeated exposure, and not after acute cocaine administration using passive IP cocaine injections (Hope *et al.* 1994, Nye *et al.* 1995; Chen *et al.* 1995). Similarly, we found that chronic IV cocaine exposure increased ΔFosB in all striatal subregions examined, regardless of whether it was administered in a volitional or passive fashion. However, a major difference from previous studies is that acute cocaine administration increased ΔFosB protein levels in both NAc core and CPu, and approached significance in the NAc shell ($p < 0.1$). One possible explanation for this difference may be the dose and/or duration of cocaine exposure, as rats in the AY group received multiple IV cocaine infusions over the single 4 h session resulting in total cocaine intake that ranged from 25.5 to 57.5 mg/kg across individual animals, which far exceeds doses of 10-20 mg/kg typically used with a single bolus IP injection (Hope *et al.* 1994; Lee *et al.* 2006). In addition, cocaine was administered via a more direct IV route of administration which produces higher peak brain levels of cocaine and dopamine that persist throughout the session, whereas these effects typically wane within an hour after IP injection (Bradberry, 2002). Thus, the ability of ΔFosB to accumulate after a single acute exposure to cocaine is likely dependent on both the strength and duration of the cocaine stimulus used in the present study. In any event, the finding that ΔFosB can accumulate after a single exposure to cocaine indicates that ΔFosB could exert its effects more rapidly than previously thought, possibly resulting from an initial self-administration binge.

Interestingly, the amount of ΔFosB accumulation differed between dorsal and ventral striatal regions over the course of chronic cocaine administration. In the NAc core, the amount of ΔFosB found immediately after the final day of chronic administration (0 h WD) was more than double the amount that was found after acute administration, and smaller ΔFosB

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increases in the NAc shell reached significance only after chronic administration, regardless of whether cocaine was self-administered or received by passive yoked infusion. Increases with chronic cocaine administration probably reflect the accumulation of highly stable ΔFosB protein since they persisted for at least 24 hours after the last exposure. In contrast, large increases in the amount of ΔFosB in the CPu failed to differ with acute or chronic exposure, potentially reflecting a ceiling produced by acute exposure in this brain region. However, even in the CPu, the accumulation of ΔFosB protein likely contributed to persistently increased ΔFosB levels after chronic exposure, since substantial tolerance developed to cocaine-induced mRNA for ΔFosB in all 3 brain regions with chronic administration.

Acute administration of IV cocaine also increased full length FosB protein levels, with greater increases in the CPu and NAc shell than NAc core. However, mRNA for FosB was induced by almost 10 fold in the NAc shell, and less than 5 fold in CPu and NAc core. Substantial tolerance developed to cocaine's ability to induce both mRNA and protein for FosB with chronic administration, although a lower induction of FosB protein remained and could potentially compete with ΔFosB for AP-1 binding partners. The relative ratio of FosB/ΔFosB mRNA also was reduced by acute cocaine administration due to relatively greater induction of ΔFosB, consistent with previous reports using amphetamine (Alibhai *et al.* 2007). In contrast to previous findings with repeated amphetamine treatments, the reduction in the relative ratio of FosB/ΔFosB mRNA by acute cocaine remained after chronic administration, reflecting the relatively higher residual induction of ΔFosB than FosB.

The fact that ΔFosB levels increase after even acute cocaine using patterns and duration of administration more typical of human intravenous drug use has important implications for the addiction process. Thus, ΔFosB could contribute to AP-1 binding activity with initial cocaine use if adequate doses were self-administered. However, ΔFosB would compete with both FosB and cFos for AP-1 binding activity, leading to downstream gene expression and neuroplasticity that is distinct from chronic administration when ΔFosB is elevated with substantially reduced cFos and FosB. Hence, ΔFosB may have greater effects after chronic cocaine administration due to both greater accumulation in the ventral striatum and decreased competition for AP-1 binding partners in both dorsal and ventral striatum. Given that striatal-specific over-expression of ΔFosB increases the motivation for cocaine (Colby *et al.* 2003), such rapid accumulation of ΔFosB with initial cocaine exposure could perpetuate cocaine use in very early stages of the addiction process. Moreover, such prominent and widespread ΔFosB expression throughout the striatum with acute exposure would alter AP-1 binding activity in a manner that could facilitate the formation of compulsive habits through early engagement of dorsal striatal circuits (Belin and Everitt, 2008).

Considering the stability of the ΔFosB isoforms, ΔFosB levels remained markedly elevated 24 hours after the last cocaine administration session, consistent with previous studies using chronic intravenous cocaine administration (Pich *et al.* 1997; Perotti *et al.* 2008). Other studies using passive experimenter administration of IP cocaine injections found that ΔFosB accumulation can persist for 1-2 weeks of withdrawal (Hope *et al.* 1994; Brenhouse and Stellar, 2006; Lee *et al.* 2006), although we found no evidence for these changes 3 weeks after cessation of cocaine administration. Together, these studies suggest that ΔFosB accumulation may persist for relatively short withdrawal periods (< 3 weeks), and directly contribute to ongoing cocaine use, but may not directly contribute to a greater propensity for relapse in prolonged withdrawal. However, ΔFosB immunoreactivity has been detected in D1 receptor-containing striatal neurons after 30 days withdrawal from repeated cocaine in mice (Lee *et al.* 2006). Such cell-specific sampling may be more sensitive to residual ΔFosB accumulation than whole tissue analysis used in the present study, or perhaps ΔFosB

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changes merely persist longer in mice than in rats. It also is possible that ΔFosB induces a cascade of transcriptional events leading to long-lasting morphological changes such as dendritic spine formation in D1-containing striatal neurons (Lee *et al.* 2006; Maze *et al.* 2010). In this regard, several ΔFosB targets including Cdk5 and NFκB are increased after chronic cocaine, and these factors may modify nucleus accumbens circuitry through changes in neuronal structural and/or function (Ang *et al.* 2001; Benavides and Bibb, 2004; Nestler, 2008). Thus, it is possible that sustained ΔFosB accumulation during withdrawal is not necessary for its long-lasting impact on future drug-taking or -seeking behavior, but instead could represent a "molecular switch" that triggers multiple cellular processes that facilitate the transition to more addicted biological states (Nestler *et al.* 2001).

The present study found that cocaine-mediated ΔFosB accumulation is not influenced by the volitional control of cocaine intake in self-administering animals consistent with previous studies using immunohistochemical procedures and multiple drugs of abuse (Perotti *et al.* 2008; Pich *et al.* 1997). This indicates that cocaine-induced increases in ΔFosB and FosB are likely related to the pharmacological response to cocaine or other downstream events of monoaminergic receptor signaling. In contrast to ΔFosB, we found that the development of tolerance to cocaine-induced cFos was substantially influenced by volitional control over cocaine intake in the NAc, but not in the CPu. Thus, tolerance to cocaine-induced cFos in the NAc failed to occur in animals receiving cocaine passively by chronic yoked infusion when compared to acute yoked infusion. These findings differ markedly from numerous reports of tolerance to psychostimulant-induced cFos in the NAc when drugs are given by passive IP injection (Hope *et al.* 1994; Nye *et al.* 1995; Chen *et al.* 1995, 1997; Alibhai *et al.* 2007). Given that tolerance to cFos in cocaine self-administering animals parallels several studies with repeated IP injections, the lack of tolerance with chronic intravenous yoked administration may be related to the stress associated with multiple and unpredictable yoked cocaine injections (Goeders 1997). The loss of tolerance in ventral rather than dorsal striatum would be consistent with a selective effect on limbic circuits involved in motivational and emotional responses. In addition, while tolerance to induction of cFos did occur in animals self-administering cocaine, there remained a substantial ~50% increase in cFos protein in the NAc shell immediately after their final self-administration session, and a trend ($p < 0.1$) for cFos increases also occurred in the core. Reasons for this discrepancy likely reflect differences between IP injection and multiple IV infusions over a 4 h period as discussed above. The residual induction of cFos in the NAc after chronic cocaine self-administration is a novel finding that forces the reconsideration of its role in the addiction process, whereby AP-1 complexes containing cFos, ΔFosB and FosB would all coexist to some extent after chronic exposure.

Given recent evidence that cFos is directly down-regulated by ΔFosB accumulation in dorsal striatum (Renthal *et al.* 2008), it is interesting that cocaine-induced cFos in the CPu was paralleled by increases in ΔFosB with acute cocaine exposure. One possibility is that the accumulation of ΔFosB with acute administration occurs too late in the 4 h session to affect cFos induction, while its presence 24 h after cocaine in chronically treated animals impedes the induction of cFos with subsequent cocaine exposure. This idea is consistent with a trend ($p = 0.067$) for a moderate positive correlation between cFos and ΔFosB levels in the CPu with acute cocaine administration (0 h WD). This notion also is consistent with the strong positive correlation between cFos induction and cocaine intake in the CPu of acute yoked animals. These findings suggest that, similar to ΔFosB, the cFos response may reflect the dose of cocaine that was received. However, in the NAc, the greater accumulation of ΔFosB with chronic yoked cocaine administration cannot account for the lack of tolerance in the cFos response in these animals. Moreover, although tolerance to cFos induction was evident in self-administering animals, the strong positive correlation between residual cFos and ΔFosB levels in the NAc shell after 24 h withdrawal does not support a negative interaction

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between cFos and ΔFosB in the ventral striatum. Another difference from the CPu data is that cFos in the NAc core was negatively rather than positively correlated with cocaine intake immediately after acute cocaine administration, which could reflect a within-session tachyphylaxis that occurs with higher dose exposure in the ventral striatum.

Overall, the findings from the present study indicate that cFos, FosB, and ΔFosB undergo distinct regional patterns of expression after acute and chronic intravenous cocaine administration. These expression patterns are uniquely dependent on both the duration and amount of drug exposure, and tolerance to cocaine-induced cFos is highly dependent on volitional cocaine self-administration. The results also show that ΔFosB can accumulate with both acute and chronic cocaine administration by intravenous injection, supporting the idea that ΔFosB accumulation may be important in early processes that promote increased cocaine-seeking behavior and contribute to the development of cocaine addiction. Ultimately, it will be important to understand how ΔFosB can indirectly influence persistent drug craving in withdrawal via relatively short-term influences on gene expression during cocaine use and early withdrawal periods. Efforts to identify the various downstream targets and their effects on neuronal morphology and/or function will ultimately clarify the role of ΔFosB and other Fos-related antigens in the expression of addictive behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used

CPu	caudate-putamen
NAc	nucleus accumbens
AY	acute yoke
CY	chronic yoke
CSA	cocaine self-administration
WD	withdrawal
IV	intravenous
IP	intraperitoneal.

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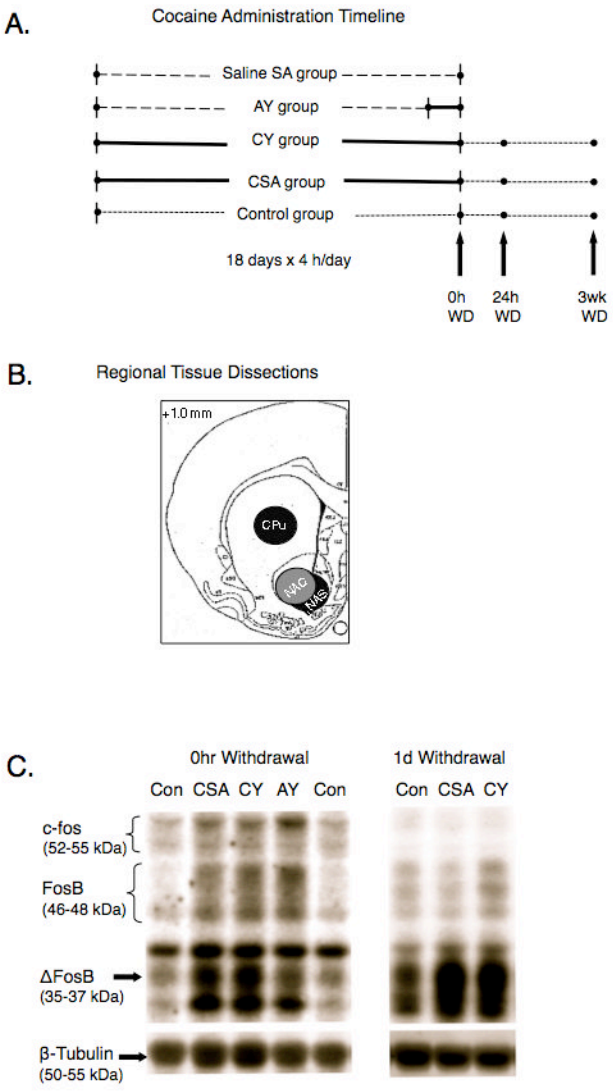

Figure 1.

(A) Timeline depicting cocaine administration and withdrawal (WD) regimens. Solid lines indicate intravenous administration of cocaine infusions (0.5 mg/kg/infusion) in chronic cocaine self-administering (CSA) and chronic yoked (CY) animals for a total of 18 days, while dashed lines indicate intravenous administration of saline in saline self-administering (saline SA) animals. Acute yoked (AY) animals received saline infusions (dashed lines) on all but the final session. Dotted lines indicate no drug availability while in the home cage (i.e., control group and during WD). Tissue was collected immediately after the final session (0 h WD), and after 24 h or 3 weeks WD. (B) Illustration depicting the posterior side of 1.5 mm thick coronal brain slices at 1.0 mm anterior to bregma (Paxinos and Watson, 1998) and the localization of tissue dissections for the NAc core (NAC), NAc shell, (NAS) and caudate-putamen (CPu). (C) Representative western blot from NAc core illustrating the molecular weights of the protein bands used to quantify levels of cFos (52-58 kDa), FosB (46-50 kDa), and ΔFosB (35-37 kDa). Bands were normalized to β-tubulin (50-55kDa).

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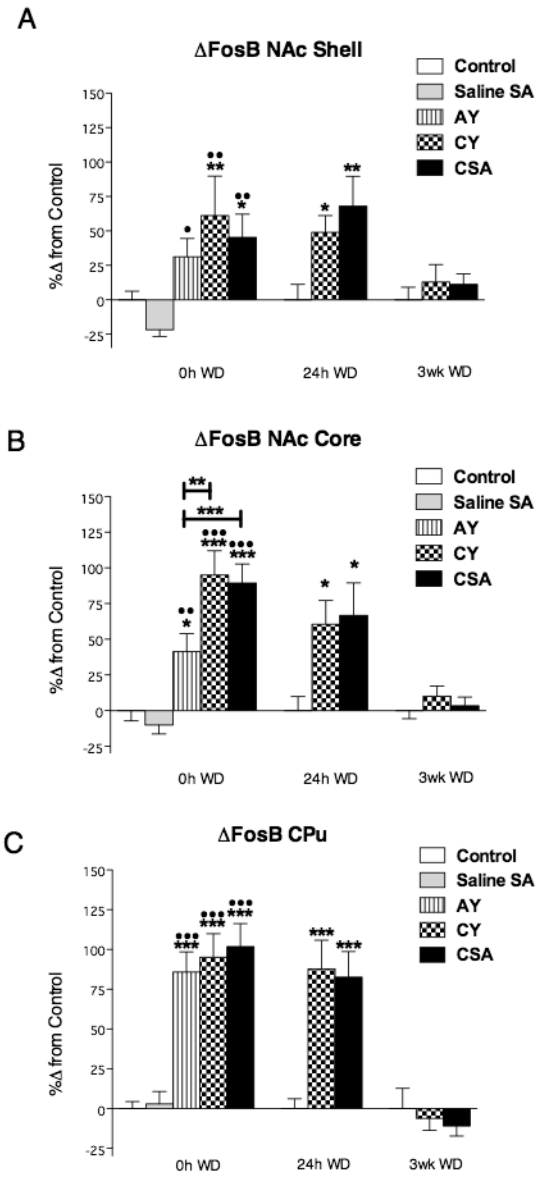

Figure 2.

Regulation of ΔFosB immediately after cocaine administration and at 24 h and 3 weeks WD. Levels of ΔFosB (35-37 kDa) are expressed as the mean ± S.E.M percent change from untreated homecage controls (Control). Tissue from saline SA animals served as a procedural control for surgery and handling. On the final session, acute yoked (AY) animals received cocaine for the first time, while chronic cocaine yoked (CY) animals received an identical number of cocaine infusions determined by the chronic cocaine self-administering (CSA) animals. Tissue samples were taken from the NAc shell (A), NAc core (B), and CPu (C). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to homecage control unless otherwise indicated by bars. •$p < 0.05$, ••$p < 0.01$, •••$p < 0.001$ compared to saline SA group at 0h WD. Sample sizes at 0 h WD; controls (n = 19-24), saline SA (n = 8), cocaine groups (n = 11-17), at 24 h WD; controls (n = 11-12), cocaine groups (n = 10-12), at 3 wk WD; controls (n = 13), cocaine groups (n = 11-12).

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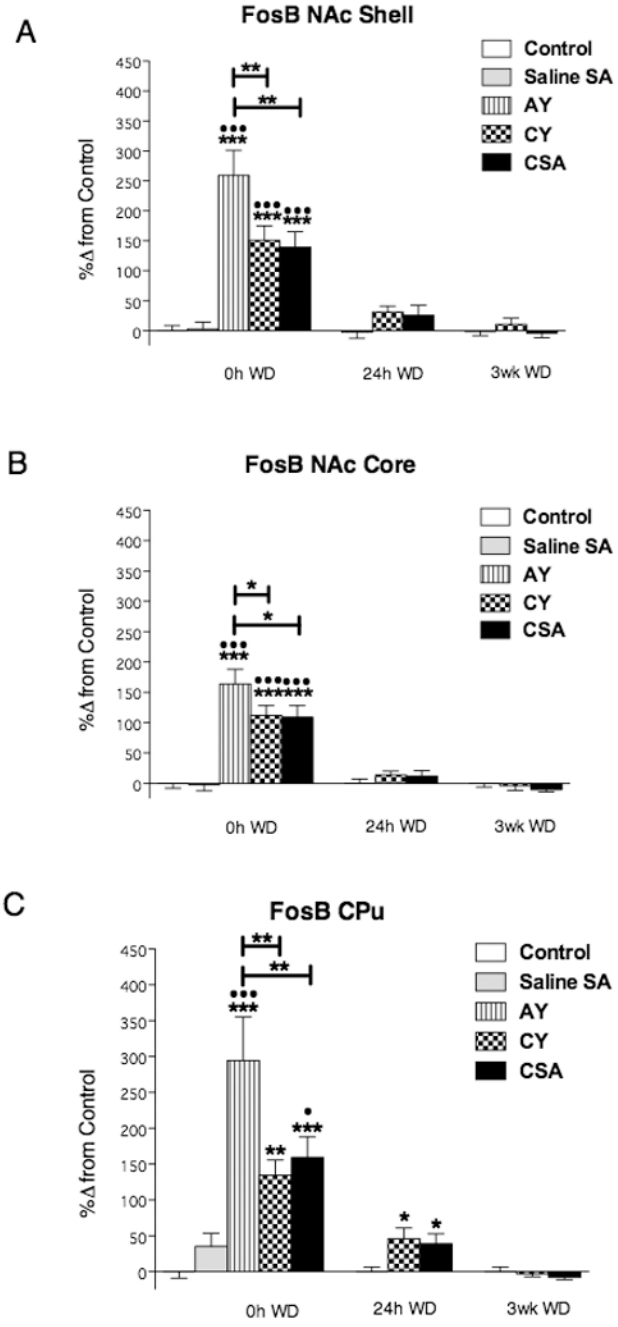

Figure 3.

Regulation of FosB immediately after cocaine administration and at 24 h and 3 weeks WD. Protein levels of FosB (46-50 kDa) are expressed as the mean ± S.E.M percent change from untreated homecage controls (see Figure 2 legend for abbreviations) in the NAc shell (A), NAc core (B), and CPu (C). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to home cage controls unless otherwise indicated by bars. •$p < 0.05$, •••$p < 0.001$ compared to saline SA group at 0h WD.

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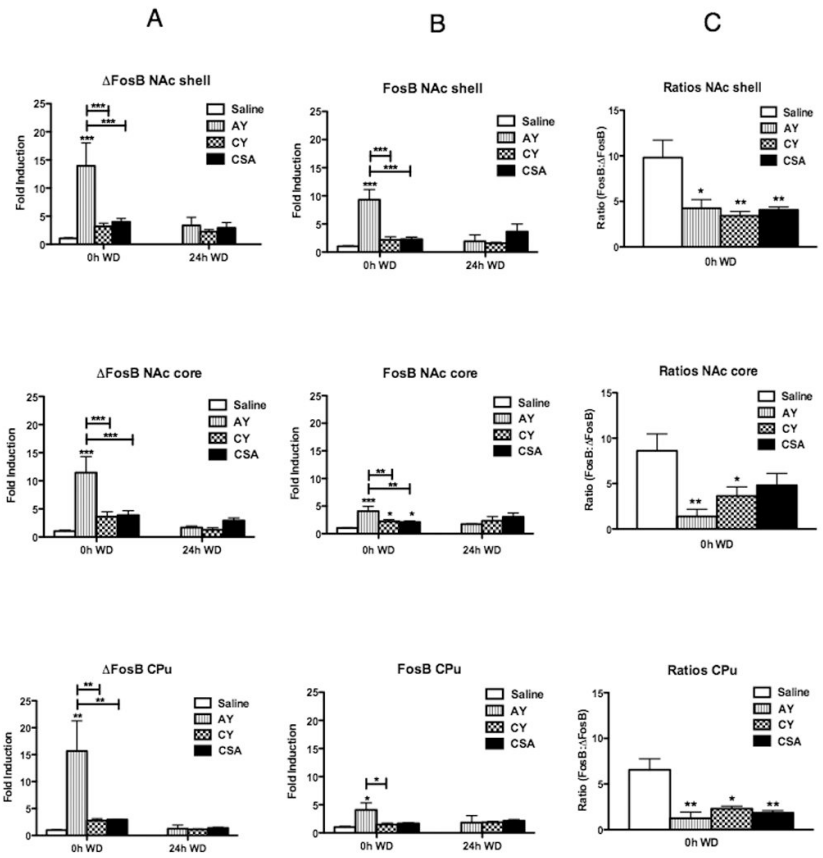

Figure 4.

Regulation of mRNA for FosB and ΔFosB immediately after cocaine administration and at 24 h WD. Quantitative RT-PCR of transcripts for ΔFosB (A), FosB (B) and the ratio FosB/ΔFosB transcripts (C) are expressed as the mean ± S.E.M fold change from saline SA controls (see Figure 2 legend for abbreviations). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to Saline SA controls unless otherwise indicated by bars. Sample sizes at 0 h WD; (n = 4-6), at 24 h WD; (n = 3-6).

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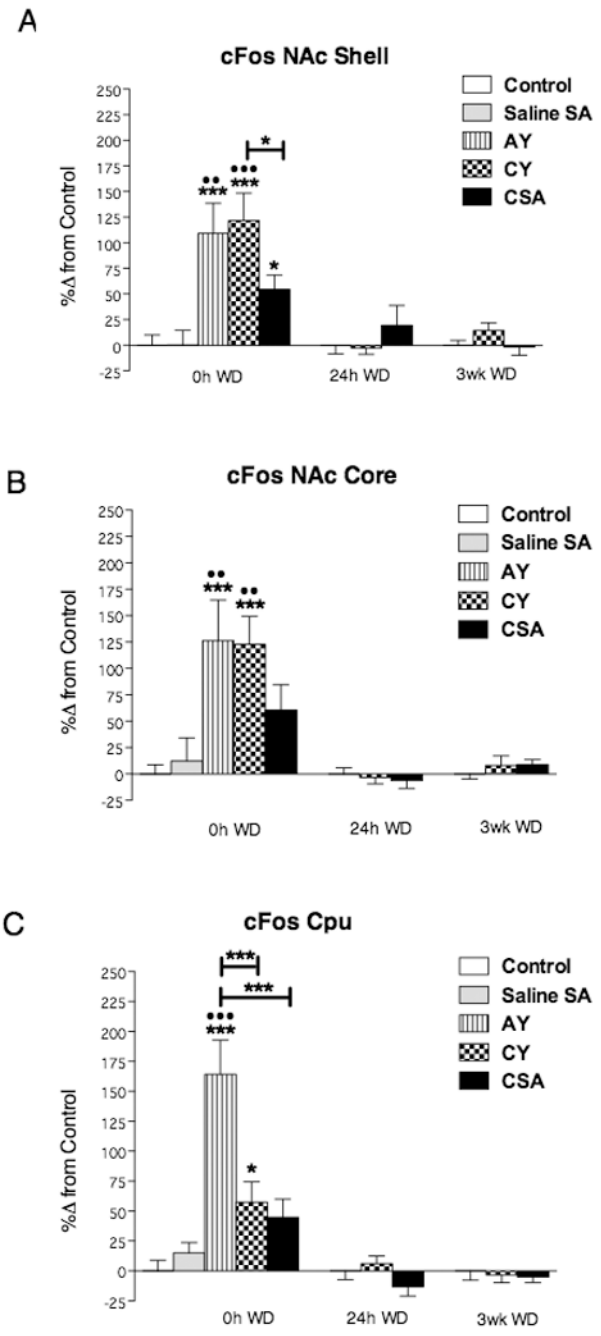

Figure 5.

Regulation of cFos immediately after cocaine administration and at 24 h WD. Protein levels of cFos (52-58 kDa) in control rats (Control, Saline SA), in rats that received passive yoked cocaine acutely (AY) or chronically (CY), and in rats that underwent chronic, active cocaine self-administration (CSA). Protein levels of cFos are expressed as the mean ± S.E.M percent change from untreated homecage controls (see Figure 2 legend for abbreviations) in the NAc shell (A), NAc core (B), and CPu (C). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to home cage controls unless otherwise indicated by bars. ••$p < 0.01$, •••$p < 0.001$ compared to saline SA group at 0h WD.

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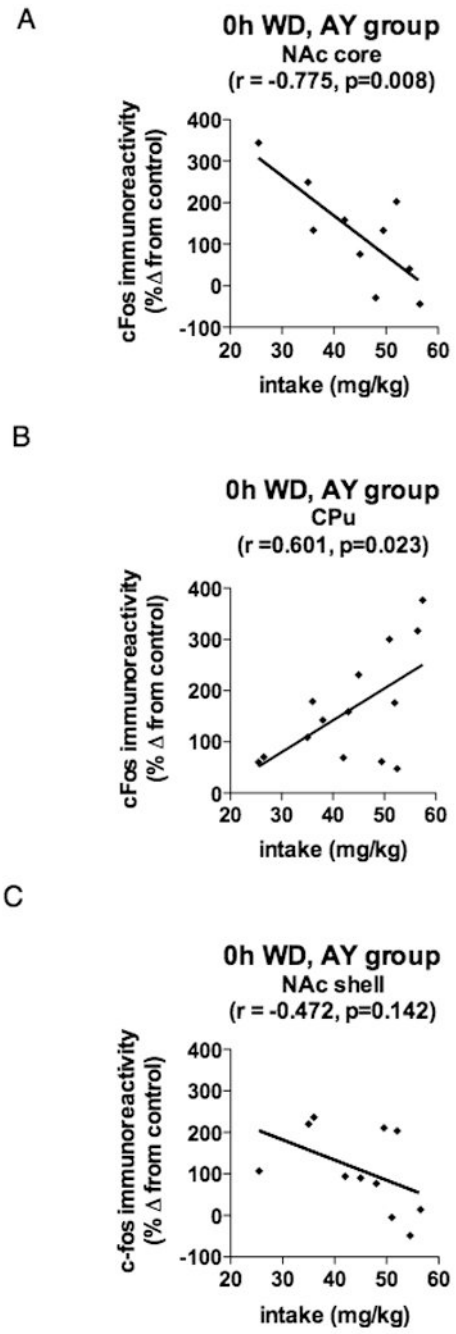

Figure 6.

Region-specific correlation between cocaine intake and cFos immunoreactivity after acute cocaine (AY). The percent increase in cFos immunoreactivity is negatively correlated with cocaine intake in the final session in the NAc core (A) and positively correlated in the CPu (B), while not significantly correlated in the NAc shell (C).

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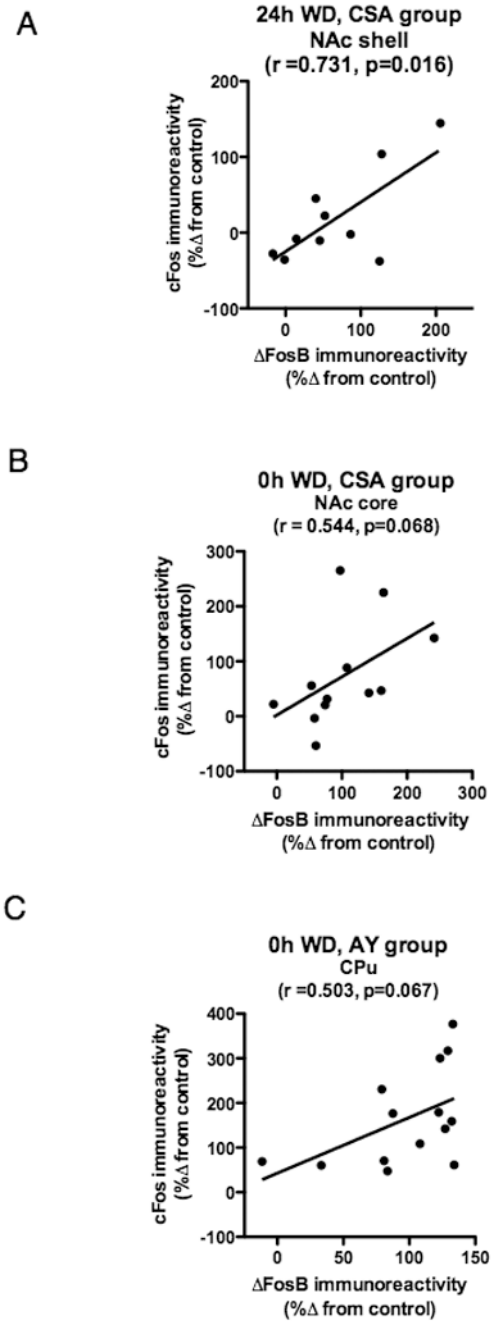

Figure 7.

Significant correlation between cFos and ΔFosB in the NAc shell in self-administering animals. The percent increase in cFos immunoreactivity is positively correlated with ΔFosB immunoreactivity after 24 h WD in cocaine self-administering animals (CSA) in the NAc shell (A), while trends for positive correlations were found immediately after cocaine administration in the NAc core in CSA animals (B), and CPu in acute yoked (AY) cocaine animals (C).