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ΔFosB induction in orbitofrontal cortex potentiates locomotor sensitization despite attenuating the cognitive dysfunction caused by cocaine

Catharine A. Winstanley *, Thomas A. Green, David E.H. Theobald, William Renthal, Quincey LaPlant [2], Ralph J. DiLeone [1], Sumana Chakravarty, Eric J. Nestler [2]

Departments of Psychiatry and Neuroscience, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9070, United States

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ABSTRACT

The effects of addictive drugs change with repeated use: many individuals become tolerant of their pleasurable effects but also more sensitive to negative sequelae (e.g., anxiety, paranoia, and drug craving). Understanding the mechanisms underlying such tolerance and sensitization may provide valuable insight into the basis of drug dependency and addiction. We have recently shown that chronic cocaine administration reduces the ability of an acute injection of cocaine to affect impulsivity in rats. However, animals become more impulsive during withdrawal from cocaine self-administration. We have also shown that chronic administration of cocaine increases expression of the transcription factor ΔFosB in the orbitofrontal cortex (OFC). Mimicking this drug-induced elevation in OFC ΔFosB through viral-mediated gene transfer mimics these behavioural changes: ΔFosB over-expression in OFC induces tolerance to the effects of an acute cocaine challenge but sensitizes rats to the cognitive sequelae of withdrawal. Here we report novel data demonstrating that increasing ΔFosB in the OFC also sensitizes animals to the locomotor-stimulant properties of cocaine. Analysis of nucleus accumbens tissue taken from rats over-expressing ΔFosB in the OFC and treated chronically with saline or cocaine does not provide support for the hypothesis that increasing OFC ΔFosB potentiates sensitization via the nucleus accumbens. These data suggest that both tolerance and sensitization to cocaine's many effects, although seemingly opposing processes, can be induced in parallel via the same biological mechanism within the same brain region, and that drug-induced changes in gene expression within the OFC play an important role in multiple aspects of addiction.

1. Introduction

The phenomena of tolerance and sensitization lie at the heart of current theories about drug addiction. In considering the Diagnostic and Statistical Manual (American Psychiatric Association DSM IV) criteria (1994) for substance abuse disorder, one of the key symptoms is that the drug user becomes tolerant to the pleasurable effects of the drug and requires more drug to achieve the same "high". However, tolerance does not develop with equal rapidity to all of a drug's effects, leading to fatal overdoses as users escalate their drug intake. Chronic drug users also become sensitized, rather than tolerant to, other aspects of the drug experience. Even though the pleasure obtained from drug intake steadily diminishes, the desire to take drug increases, and drug addicts often sensitize to negative effects of the drug (e.g., anxiety, paranoia) as well as to the power of drug-paired cues to trigger drug-craving and -seeking behaviour (Robinson and Berridge, 1993). Through understanding the biological mechanisms underpinning sensitization and tolerance to a drug, it is hoped that ways will be found to reverse or inhibit the process of addiction.

As a result, the phenomenon of locomotor sensitization has been intensively researched, particularly in laboratory rodents (see (Pierce and Kalivas, 1997) for review). Psychostimulant drugs like cocaine and amphetamine increase locomotor activity. After repeated administration, this response becomes sensitized and the animal becomes significantly more hyperactive after an acute drug challenge. It is now well-established that locomotor sensitization critically depends on changes in dopaminergic and glutamatergic signalling within the nucleus accumbens (NAc) (see (Kalivas and Stewart, 1991; Karler et al., 1994; Wolf, 1998). A plethora of molecular signalling proteins have also been identified which may contribute to the expression of this sensitized motor response. One such protein is the transcription factor ΔFosB which is increased in the NAc and dorsal striatum after chronic, but not acute, administration of numerous addictive drugs (Nestler, 2008). Increasing NAc levels of ΔFosB increases locomotor sensitization to cocaine, increases conditioned place preference to the drug, and also facilitates cocaine self-administration (Colby et al., 2003; Kelz et al., 1999). It would therefore appear that the induction of ΔFosB in the NAc facilitates the development of the addicted state.

* Corresponding author. Current address: Department of Psychology, University of British Columbia, 2136 West Mall, Vancouver BC, Canada V6T 1Z4.
E-mail address: cwinstanley@psych.ubc.ca (C.A. Winstanley).
[1] Current address: Department of Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven CT 06508, United States.
[2] Current address: Fishberg Department of Neuroscience, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029, United States.

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It is increasingly recognised that repeated exposure to addictive drugs affects higher-order cognitive functions like decision-making and impulse control, and that this has a crucial impact on relapse to drug-seeking (Bechara, 2005; Garavan and Hester, 2007; Jentsch and Taylor, 1999). Deficits in impulse control have been observed in recently abstinent cocaine addicts, as well as users of other drugs (e.g. (Hanson et al., 2008; Lejuez et al., 2005; Moeller et al., 2005; Verdejo-Garcia et al., 2007). It has been hypothesised that this impulsivity stems from hypoactivity in the orbitofrontal cortex (OFC) observed in such populations (Kalivas and Volkow, 2005; Rogers et al., 1999; Schoenbaum et al., 2006; Volkow and Fowler, 2000). We recently observed that repeated cocaine administration increases levels of ΔFosB within the OFC, and that mimicking this induction by infusing adeno-associated virus (AAV) designed to over-express ΔFosB into the OFC (viral-mediated gene transfer) appears to activate local inhibitory circuits (Winstanley et al., 2007). High levels of OFC ΔFosB may therefore theoretically contribute to drug-induced changes in impulse control.

We recently completed a series of studies to test this hypothesis, and to determine the effects of acute and chronic administration of cocaine on two measures of impulsivity in rats: the level of premature (impulsive) responding on the five-choice serial reaction time task (5CSRT) and selection of a small immediate over a larger delayed reward in a delay-discounting task (Winstanley et al., 2007). We observed that acute cocaine increased impulsive responding on the 5CSRT yet decreased impulsive choice of the small immediate reward in the delay-discounting paradigm, mimicking the effects of amphetamine. This pattern of behavior—an increase in impulsive action yet a decrease in impulsive choice—has been interpreted as an increase in incentive motivation for reward (Uslaner and Robinson, 2006). However, after repeated administration of cocaine, rats no longer showed such pronounced changes in impulsivity, as if they had become tolerant to these cognitive effects of the drug. This is in stark contrast to the sensitized locomotor response to cocaine observed after chronic administration discussed above. Furthermore, over-expression of ΔFosB in the OFC mimicked the effects of chronic cocaine treatment: the effects of acute cocaine on performance of both the 5CSRT and delay-discounting tasks was attenuated in these animals, as if they had already developed tolerance to the drugs' effects.

However, while increasing ΔFosB in the OFC prevented acute cocaine from increasing impulsivity, this same manipulation actually increased impulsivity during withdrawal from a long-access cocaine self-administration regime (Winstanley et al., 2008). The cognitive performance of these animals was therefore less affected when cocaine was on-board, yet they were more vulnerable to impulse control deficits during withdrawal. The same manipulation—increasing ΔFosB in the OFC—can therefore increase tolerance or sensitivity to aspects of cocaine's effects. Here we report novel additional data showing that animals which showed a blunted response to an acute cocaine challenge in the impulsivity tests following over-expression of ΔFosB in the OFC were also sensitized to the locomotor stimulant actions of cocaine. Thus, tolerance and sensitization to different aspects of cocaine's effects were observed in the same subjects. Given the pronounced role of the NAc in mediating locomotor sensitization, and the absence of data implicating the OFC in motor regulation, we hypothesised that increasing ΔFosB in the OFC may have enhanced the motor response to cocaine through altering function in this striatal region. We therefore conducted a separate experiment using real-time PCR to investigate whether increasing ΔFosB in the OFC alters gene expression in the NAc in a manner indicative of enhancing locomotor sensitization.

2. Methods

All experiments were carried out in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at UT Southwestern.

2.1. Subjects

Male Long Evans rats (initial weight: 275–300 g; Charles River, Kingston, RI) were housed in pairs under a reverse light cycle (lights on from 21.00–09.00) in a climate-controlled colony room. Animals in the behavioral experiment ($n=84$) were food restricted to 85% of their free-feeding weight and maintained on 14 g of rat chow per day. Water was available *ad libitum*. Behavioral testing took place between 09.00 and 19.00 five days per week. Animals used to generate brain tissue for the qPCR experiments had free access to both food and water ($n=16$). These animals had free access to both food and water.

2.2. Surgery

Rats received intra-OFC injections of either AAV-GFP, AAV-ΔFosB, or AAV-ΔJunD using standard stereotaxic techniques as described (Winstanley et al., 2007). Rats were anaesthetised with ketamine (Ketaset, 100 mg/kg intramuscular (i.m.) injection) and xylazine (10 mg/kg i.m.; both drugs from Henry Schein, Melville, NY). AAVs were infused into the OFC using a 31 gauge stainless steel injector (Small Parts, Florida, USA) attached to a Hamilton microinfusion pump by polyethylene tubing (Instech Solomon, Pennsylvania, USA). The viral vectors were infused at a rate of 0.1 μl/min according to the following coordinates taken from a stereotaxic atlas (Paxinos and Watson, 1998): site 1 AP +4.0, L ±0.8, DV −3.4, 0.4 μl: site 2 AP +3.7, L ±2.0, DV −3.6, 0.6 μl: site 3 AP ±3.2, L ± 2.6, DV −4.4, 0.6 μl (see (Hommel et al., 2003) for details of AAV preparation). The AP (anteroposterior) co-ordinate was taken from bregma, the L (lateral) co-ordinate from the midline and the DV (dorso-ventral) co-ordinate from dura. Animals were allowed one week to recover from surgery before any behavioral testing (experiment 1) or drug administration (experiment 2) commenced.

2.3. Experimental design

The locomotor sensitization data were obtained from animals which had undergone a series of behavioural tests to measure the cognitive sequelae of chronic drug exposure, and these data have been published previously (Winstanley et al., 2007). In brief, rats were trained to perform the 5CSRT or the delay-discounting task. They were then divided into three groups matched for baseline performance. An adeno-associated virus (AAV2) over-expressing ΔFosB (Zachariou et al., 2006) was infused selectively into the OFC of one group using standard stereotaxic surgical techniques (see below) thereby mimicking the induction of this protein by chronic cocaine administration. A second group received intra-OFC infusions of AAV-ΔJunD. AAV-GFP (green fluorescent protein) was used for the control group. Once a stable post-operative baseline was established, the effects of acute cocaine (0, 5, 10, 20 mg/kg i.p.) were determined on-task. To assess whether chronic administration of cocaine alters the cognitive effects of an acute cocaine exposure, animals were then matched both within and between their surgery groups into two equal sets. One group was treated chronically with saline, the other with cocaine (2×15 mg/kg) for 21 days. Two weeks after chronic drug treatment ceased, the acute cocaine challenges were repeated on-task. One week later, the locomotor response to cocaine was assessed.

2.4. Locomotor response to cocaine

Locomotor activity was assessed in individual cages (25 cm× 45 cm×21 cm) using a photobeam activity system (PAS: San Diego Instruments, San Diego, CA). Activity in each cage was measured by 7 photobeams crossing the width of the cage, 6 cm apart and 3 cm from the cage floor. The data were collated over 5 min bins using the PAS software (version 2, San Diego Instruments, San Diego, CA). After 30 min, animals were injected with cocaine (15 mg/kg i.p.) and locomotor activity monitored for a further 60 min.

Table 1
Sequence of primers used to quantify levels of cDNA via real-time PCR.

Gene of interest	Forward	Reverse
FosB	GTGAGAGATTTGCCAGGGTC	AGAGAGAAGCCGTCAGGTTG
ΔFosB	AGGCAGAGCTGGAGTCGGAGAT	GCCGAGGACTTGAACTTCACTCG
CREB	AGTGACTGAGGAGCTTGTACCA	TGTGGCTGGGCTTGAAC
Arc	TGGGTGGAGTTCAAGAAGGA	TCTGGTACAGGTCCCGCTTA
psd95	AGACTCGGTTCTGAGCTATG	TCTTTGGTAGGCCCAAGGAT
D_2 receptor	ATGGCAAAACCCGGACCT	GAACACACCGAGAACAATGGC
$GABA_B$ receptor	CAGCCCAACCTGAACAATCT	ACAAACGGGAACTGGCTTC
GluR1	CGAGTTCTGCTACAAATCCCG	TGTCCGTATGGCTTCATTGATG
GluR2	ATTGTAGACTACGATGATTC	AATAGTCAGCTTGTACTTGA
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

All sequences are given in the 5'–3' direction. Abbreviations used: CREB: cyclic adenosine monophosphate response element binding protein; Arc: activity-related cytoskeleton-associated protein; psd95: post-synaptic density 95; GABA: gamma aminobutyric acid; GluR: glutamate receptor subunit; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

2.5. Quantification of mRNA

Rats received intra-OFC injections of AAV-GFP or AAV-ΔFosB, followed by 21 twice daily injections of saline or cocaine, exactly as described for the behavioral experiments. Animals were used 24 h after the last saline or cocaine injection. Rats were killed by decapitation. The brains were rapidly extracted and bilateral 1 mm thick 12 gauge punches of the NAc were obtained and immediately frozen and stored at −80 °C until RNA isolation. Punches from the OFC were also removed for analysis by DNA microarray which confirmed successful viral-mediated gene transfer in this region (see (Winstanley et al., 2007) for more detailed results). RNA was extracted from the NAc samples using the RNA Stat-60 reagent (Teltest, Houston, TX) according to the manufacturer's instructions. Contaminating DNA was removed with DNase treatment (DNA-Free, catalogue # 1906, Ambion, Austin TX). Purified RNA was reverse-transcribed into cDNA (Superscript First Strand Synthesis, Catalogue #12371-019; Invitrogen). Transcripts for genes of interest were quantified using real-time qPCR (SYBR Green; Applied Biosystems, Foster City, CA) on a Stratagene (La Jolla, CA) Mx5000p 96-well thermocycler. All primers were custom-synthesised by Operon (Huntsville, AL; see Table 1 for sequences) and validated for linearity and specificity before experiments. All PCR data were normalized to levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was not altered by cocaine treatment, according to the following formula: $\Delta C_t = C_t(\text{gene of interest}) - C_t$ (GAPDH). Adjusted expression levels for both the AAV-ΔFosB and AAV-GFP rats which received cocaine, and the AAV-ΔFosB rats which received chronic saline, were then calculated relative to controls (AAV-GFP group given chronic saline) as follows: $\Delta\Delta C_t = \Delta C_t - \Delta C_t$ (control group). In keeping with recommended practice in the field (Livak and Schmittgen, 2001), expression levels relative to controls were then calculated using the following expression: $2^{-\Delta\Delta Ct}$.

2.6. Drugs

Cocaine HCl (Sigma, St. Louis, MO) was dissolved in 0.9% saline in a volume of 1 ml/kg and administered via i.p. injection. Doses were calculated as the salt.

2.7. Data analysis

All data were analyzed using SPSS software (SPSS, Chicago, IL). Locomotor data were subjected to multifactorial ANOVA with surgery (two levels: GFP vs ΔFosB or ΔJunD) and chronic treatment (two levels, chronic saline and chronic cocaine) as between subjects factors, and time bin as a within subjects factor. Data from real-time PCR experiments were analysed by univariate ANOVA with surgery (two levels: GFP vs ΔFosB) and chronic treatment (two levels, chronic saline and chronic cocaine) as fixed factors. Main effects were followed up by independent samples *t*-tests where appropriate.

3. Results

Experiment 1. *Chronic cocaine administration produces sensitization to the hyperlocomotor effects of acute cocaine which is mimicked by ΔFosB.*

As would be expected, robust locomotor sensitization was observed in control animals after chronic cocaine exposure, with animals treated chronically with cocaine showing increased hyperactivity in response to the acute cocaine challenge (Fig. 1A, chronic treatment: $F_{1,34} = 4.325$, $p < 0.045$). Animals over-expressing ΔJunD, a dominant negative mutant of JunD which acts as a ΔFosB antagonist (Zachariou et al., 2006), in the OFC were indistinguishable from control animals (Fig. 1C, GFP vs ΔJunD, group: $F_{1,56} = 1.509$, NS). However, animals over-expressing ΔFosB in the OFC which had received repeated saline injections appeared "pre-sensitized": they showed an enhanced locomotor response to acute cocaine which was indistinguishable from the sensitized response of their counterparts treated with chronic cocaine (Fig. 1B, GFP vs ΔFosB surgery × chronic treatment: $F_{1,56} = 3.926$, $p < 0.052$; ΔFosB only: chronic treatment: $F_{1,22} = 0.664$, NS). ΔFosB animals were slightly hyperactive within the first 15 min of being placed in the locomotor

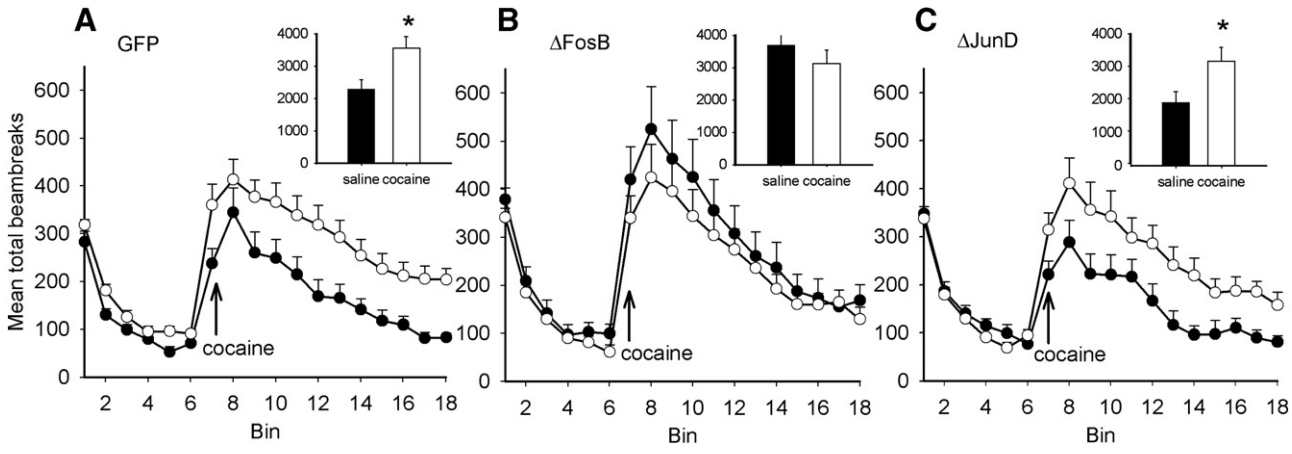

Fig. 1. Locomotor sensitization to cocaine. Acute cocaine produced greater increases in locomotor activity in control animals treated chronically with cocaine versus saline (panel A). In animals over-expressing ΔFosB (panel B), those given repeated saline injections were just as hyperactive following acute cocaine as those given repeated cocaine injections, and their activity level was comparable to sensitized control animals. Over-expression of ΔJunD did not prevent development of locomotor sensitization (panel C). Data is blocked in 5 min bins. Open circles depict the acute response to cocaine in animals previously treated with chronic cocaine; closed circles depict the acute response to cocaine in animals previously treated with chronic saline. Data shown are mean + SEM. * = $p < 0.05$.

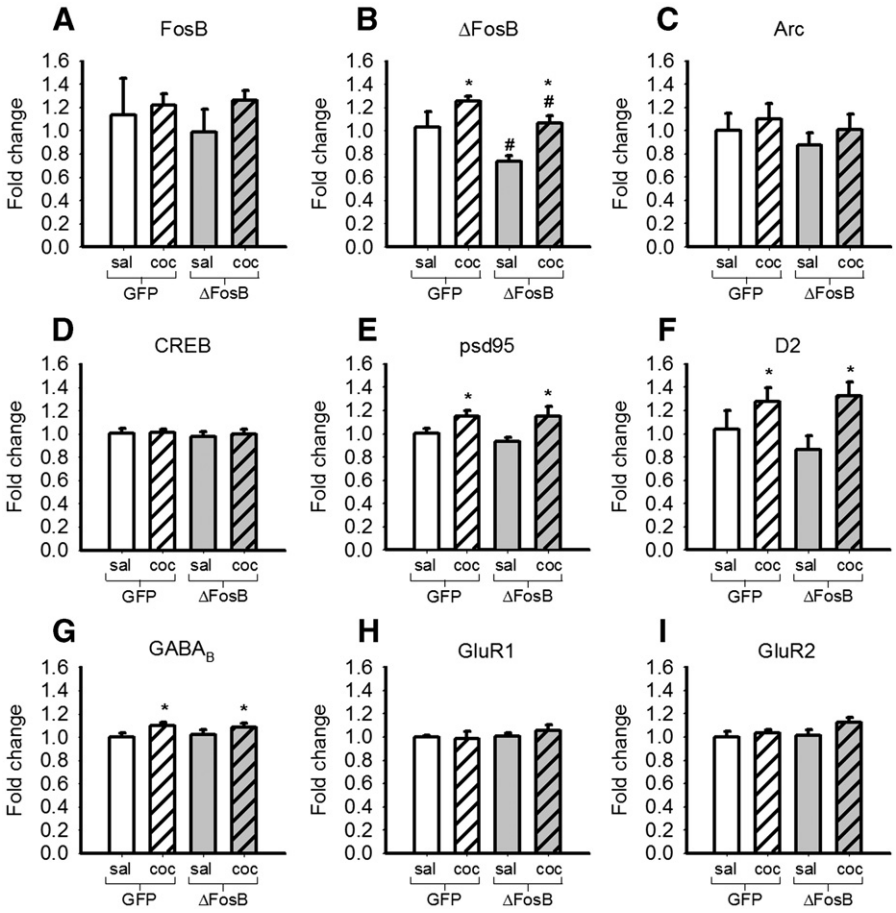

Fig. 2. Changes in mRNA within the NAc of animals over-expressing either GFP or ΔFosB in the OFC, and treated chronically with either saline or cocaine. Data indicate linear fold changes in expression as a proportion of control values. Data shown are mean + SEM. * = $p<0.05$, main effect of cocaine treatment; # = $p<0.05$ main effect of over-expressing ΔFosB relative to GFP.

boxes (GFP vs ΔFosB, surgery: $F_{1,56}=4.229$, $p<0.04$), but levels of locomotor activity were comparable to controls in the 15 min prior to cocaine administration (surgery: $F_{1,56}=0.138$, NS).

Considering that, when given cocaine during the 5CSRT, the same animals showed a relatively enhanced ability to withhold from making premature motor responses, this hyperactivity appears specific to ambulatory locomotion i.e. the kind of movement which is typically recorded in locomotor sensitization studies. Although enhanced activity in response to stimulant drugs could reflect an anxiogenic profile, intra-OFC over-expression of ΔFosB does not increase anxiety as measured using the elevated plus maze or open field test (data not shown). The animals were also well-habituated to IP injections, and saline injections did not alter their cognitive performance (Winstanley et al., 2007), therefore this motor effect cannot be attributed to a general response to an IP injection. In summary, these findings indicate that induction of ΔFosB in the OFC is sufficient (but not necessary) for sensitized locomotor responding to cocaine, even though ΔFosB in the same region causes tolerance to the effects of cocaine on motivation and impulsivity (Winstanley et al., 2007).

Experiment 2. *Chronic cocaine administration modulates gene expression in the NAc.*

If a particular molecule in the NAc was contributing to the pre-sensitized response seen in the AAV-ΔFosB saline-treated group, then we would expect to see a similar biochemical response in these animals when compared to animals in both the AAV-GFP and AAV-ΔFosB groups treated chronically with cocaine. Furthermore, animals in the AAV-GFP group treated with saline should not show this response as these animals are not sensitized to cocaine. This pattern of results would be reflected in a significant drug × surgery interaction, supported by a significant independent samples *t*-test comparing the means of the AAV-GFP and AAV-ΔFosB saline treated groups, plus the AAV-ΔFosB and AAV-GFP cocaine treated groups. Main effects of drug treatment or surgery would confirm that chronic cocaine or over-expression of ΔFosB in the OFC could modulate the target molecule in the NAc, but this observation is insufficient to explain the sensitized locomotor response observed in the AAV-ΔFosB saline treated group. Tissue from one animal which received intra-OFC infusions of AAV-GFP and repeated cocaine injections could not be analysed due to unusually low yield of RNA. In this experiment, we focused on several genes which have been implicated in locomotor sensitization to cocaine (see Discussion).

3.1. ΔFosB/FosB

Levels of FosB mRNA in the NAc were not altered by either chronic drug treatment (Fig. 2A, drug: $F_{1,14}=1.179$, n.s.) or expression of ΔFosB in the OFC (surgery: $F_{1,14}=0.235$, n.s.). However, levels of ΔFosB were significantly higher in animals treated chronically with cocaine in accordance with previous reports (Chen et al., 1997); Fig. 2B, drug: $F_{1,14}=7.140$, $p<0.022$). Interestingly, the amount of ΔFosB mRNA in the NAc of saline-treated animals was lower in those in which this transcription factor had been over-expressed in the OFC (drug: $F_{1,14}=9.362$, $p<0.011$). However, the absence of a drug × surgery interaction indicates that chronic cocaine treatment was having the same effect in both AAV-GFP and AAV-ΔFosB treated groups, proportionally elevating ΔFosB levels to a similar extent (drug × surgery: $F_{1,14}=0.302$, n.s.).

3.2. Arc/CREB/PSD95

There was no evidence of increased Arc (activity-related cytoskeleton-associated protein) expression 24 h after the last drug exposure,

nor did increasing ΔFosB in the OFC change levels of Arc mRNA in the NAc (Fig. 2C, drug: $F_{1,14}=1.416$, n.s.; surgery: $F_{1,14}=1.304$, n.s.). Similarly, no changes were observed in CREB (cAMP response element binding protein) expression (Fig. 2D, drug: $F_{1,14}=0.004$, n.s.; surgery: $F_{1,14}=0.053$, n.s.). However, chronic administration of cocaine significantly increased mRNA levels for PSD95 (postsynaptic density protein of 95 kD) (Fig. 2E, drug: $F_{1,14}=11.275$, $p<0.006$), but this increase was similar in both AAV-GFP and AAV-ΔFosB groups (surgery: $F_{1,14}=0.680$, n.s.; drug × surgery: $F_{1,14}=0.094$, n.s.).

3.3. D_2 /$GABA_B$ /GluR1/GluR2

Levels of mRNA for dopamine D_2 receptors increased following chronic cocaine administration (Fig. 2F, drug: $F_{1,14}=7.994$, $p<0.016$), but this increase was unaffected by over-expression of ΔFosB in the OFC (surgery: $F_{1,14}=0.524$, n.s.; drug × surgery: $F_{1,14}=0.291$, n.s.). mRNA levels of the $GABA_B$ receptor showed a similar profile, with levels increasing by a small yet significant amount following repeated exposure to cocaine regardless of the viral manipulation (Fig. 2G, drug: $F_{1,14}=5.644$, $p<0.037$; surgery: $F_{1,14}=0.000$, n.s.; drug × surgery: $F_{1,14}=0.463$, n.s.). However, levels of the AMPA glutamate receptor subunits GluR1 and GluR2 were not affected by any manipulation, although there was a slight trend for an increase in GluR2 following chronic cocaine treatment (Fig. 2H, GluR1: drug: $F_{1,14}=0.285$, n.s; surgery: $F_{1,14}=0.323$, n.s.; drug × surgery: $F_{1,14}=0.224$, n.s.; Fig. 2I, GluR2: drug: $F_{1,14}=3.399$, $p<0.092$; surgery: $F_{1,14}=0.981$, n.s.; drug × surgery: $F_{1,14}=0.449$, n.s.).

In summary, although chronic cocaine treatment altered mRNA levels for a number of the genes tested in the NAc, we did not see a corresponding increase in expression of these genes in saline-treated rats over-expressing ΔFosB in the OFC. These findings suggest that these particular genes are not involved in the increased locomotor response observed in this group.

4. Discussion

Here we show that over-expression of ΔFosB in the OFC sensitized rats to the locomotor stimulant actions of cocaine, mimicking the actions of chronic cocaine administration. We have previously shown that the performance of these same animals on the 5CSRT and delay-discounting paradigms is less affected by acute cocaine, and that a similar tolerance-like effect is observed after repeated cocaine exposure. Thus, sensitization and tolerance to different actions of cocaine can be observed in the same animals, with both adaptations mediated via the same molecule, ΔFosB, acting in the same brain region. The fact that both phenomena can be concurrently induced by mimicking one of the actions of cocaine at a single frontocortical locus highlights the importance of cortical regions in the sequelae of chronic drug intake. Furthermore, these data suggest that tolerance and sensitization reflect two seemingly contrasting, yet intimately related, aspects of the response to addictive drugs.

Given that increased ΔFosB expression in the NAc is critically involved in the development of locomotor sensitization, one plausible hypothesis would have been that over-expressing ΔFosB in the OFC pre-sensitizes animals to cocaine by increasing levels of ΔFosB in the NAc. However, the inverse result was found: levels of ΔFosB in the NAc were significantly lower in animals over-expressing ΔFosB in the OFC. The behavioural consequences of this decrease in NAc ΔFosB are hard to interpret, as inhibiting ΔFosB's actions through over-expression of ΔJunD in this region reduces many of cocaine's effects in mice (Peakman et al., 2003). Certain parallels exist between these observations and those made in reference to the dopamine system. For example, partial dopamine depletion in the NAc can lead to hyperactivity as can direct application of dopamine agonists in this region (Bachtell et al., 2005; Costall et al., 1984; Parkinson et al., 2002; Winstanley et al., 2005b). Likewise, the fact that increasing cortical levels of ΔFosB may decrease subcortical expression resembles the well-established finding that an increase in prefrontal dopaminergic transmission is often accompanied by a reciprocal decrease in striatal dopamine levels (Deutch et al., 1990; Mitchell and Gratton, 1992). How such a feedback mechanism may work for intra-cellular signalling molecules is currently unclear, but may reflect changes in the general activity of certain neuronal networks caused by a change in gene transcription. For example, increasing ΔFosB in the OFC leads to an upregulation of local inhibitory activity, as evidenced by an increase in levels of the $GABA_A$ receptor, mGluR5 receptor and substance P, as detected by microarray analysis (Winstanley et al., 2007). This change in OFC activity could then affect activity in other brain areas, which could in turn lead to a local change in expression of ΔFosB. Whether levels of ΔFosB reflect relative changes in dopamine activity is an issue that warrants further investigation.

All animals showed a significant increase in ΔFosB mRNA levels in the NAc following chronic cocaine treatment, in keeping with previous reports of increased protein levels (Chen et al., 1997; Hope et al., 1992; Nye et al., 1995). However, a recent report found that levels of ΔFosB mRNA were no longer significantly elevated 24 h after chronic amphetamine treatment, although significant increases were observed 3 h after the final injection (Alibhai et al., 2007). This discrepancy may be due to the difference in the psychostimulant drug used (cocaine vs amphetamine), but given the shorter half-life of cocaine, it would be reasonable to expect that its effects on gene expression would normalise more rapidly than those of amphetamine, rather than vice versa. A more plausible reason for these different results is that animals in the current study were injected with a moderate dose of drug twice daily for 21 days compared to a single high dose injection for 7 days (Alibhai et al., 2007). The more extended regimen of treatment could have resulted in the more pronounced changes observed here.

Although the changes in gene expression observed within the NAc following chronic cocaine are in general agreement with previously reported findings, the magnitude of the effects is smaller in the current study. One potential reason for this is that animals were sacrificed only 24 h after the last injection of cocaine, whereas the majority of studies have used tissue obtained two weeks since the last drug exposure. Studies exploring the time-course of locomotor sensitization indicate that more pronounced changes in both behaviour and gene/protein expression are observed at this later time-point. Although we report a slight increase in mRNA for the dopamine D_2 receptor in the NAc, the general consensus is that expression levels of the D_2 or D_1 receptor are not permanently altered following development of locomotor sensitization, although both increases and decreases in D_2 receptor number have been reported shortly after the end of the sensitizing regime (see (Pierce and Kalivas, 1997) for discussion). Our observation that GluR1 and GluR2 mRNA were unchanged following chronic cocaine treatment at this early time-point is likewise in accordance with a previous report (Fitzgerald et al., 1996), although an increase in GluR1 mRNA has been detected at later time-points after the cessation of chronic psychostimulant treatment (Churchill et al., 1999).

However, we did observe a small increase in PSD95 mRNA in the NAc of animals treated chronically with cocaine. PSD95 is a scaffolding molecule, and is one of the major proteins within the postsynaptic density of excitatory synapses. It anchors several glutamate receptors and associated signaling proteins at the synapse, and an increase in PSD95 expression is thought to reflect increased synaptic activity and increased insertion and stabilization of glutamate receptors at synapses (van Zundert et al., 2004). A role for PSD95 in the development of locomotor sensitization has been suggested previously (Yao et al., 2004).

Increases in Arc expression have also been linked to increases in synaptic activity. However, while an increase in Arc expression in the NAc has been observed 50 min after injection with amphetamine (Klebaur et al., 2002), our data indicate that chronic administration of cocaine does not upregulate Arc in the NAc more permanently, although increases in Arc have been observed 24 h after chronic dosing with antidepressant drugs (Larsen et al., 2007) and amphetamine (Ujike et al., 2002). An increase in CREB phosphorylation is

also observed in the NAc after acute cocaine and amphetamine administration (Kano et al., 1995; Konradi et al., 1994; Self et al., 1998), but it is perhaps not surprising that no increase in CREB mRNA was observed following chronic cocaine administration. Signaling through the CREB pathway is thought to be more important in the initial phases of drug-taking, with transcription factors such as ΔFosB coming to dominate as addiction progresses (McClung and Nestler, 2003). Although CREB has been implicated in the rewarding effects of cocaine (Carlezon et al., 1998), there have been no reports that increasing CREB expression affects locomotor sensitization, although viral-mediated increases in the endogenous dominant negative antagonist of CREB, the inducible cAMP early repressor protein or ICER, increases hyperactivity caused by an acute injection of amphetamine (Green et al., 2006).

In summary, although the majority of the drug-induced changes we observed are concordant with predictions from the literature, we did not find any changes in gene expression within the NAc which could explain the sensitized locomotor response to cocaine observed in drug-naïve animals treated with intra-OFC AAV-ΔFosB. This raises the possibility that increasing ΔFosB in the OFC may not be affecting motor sensitization via the NAc, although many other genes, not studied here, could possibly be involved. Considerable evidence suggests that modulation of the medial prefrontal cortex (mPFC) can change striatal activity and thereby contribute to behavioral sensitization to psychostimulants (Steketee, 2003; Steketee and Walsh, 2005), although less is known about the role of more ventral prefrontal regions like the OFC. The NAc receives some projections from the OFC (Berendse et al., 1992). However, a more recent and detailed study identified very few direct OFC-NAc projections: sparse labelling of the most lateral part of the NAc shell was observed following injections of anterograde tracer into the lateral and ventrolateral areas of the OFC, and the most ventral OFC region sends minimal projections to the NAc core (Schilman et al., 2008). The central caudate-putamen receives much denser innervation. In light of this anatomical evidence, the majority of the NAc tissue analysed in our PCR reactions would not have been directly innervated by the OFC, decreasing the chances that any changes in gene expression would be successfully detected.

The OFC does project heavily to regions which themselves are strongly connected with the NAc, such as the mPFC, basolateral amygdala (BLA), caudate putamen and subthalamic nucleus (STN). Whether changes in the OFC could indirectly modulate functioning of the NAc through its influence in these areas is an open question. It has been shown that activity in the BLA is altered after OFC lesions, and that this significantly contributes to the deficits in reversal learning caused by OFC damage (Stalnaker et al., 2007), but any effects within areas such as the NAc have yet to be reported. It may be more productive to focus attention on other areas more strongly connected to the OFC and which are also heavily implicated in motor control. The STN is a particularly promising target, as not only do lesions of the STN and OFC produce similar effects on impulsivity and Pavlovian learning (Baunez and Robbins, 1997; Chudasama et al., 2003; Uslaner and Robinson, 2006; Winstanley et al., 2005a), but psychostimulant-induced locomotor sensitization is associated with an increase in c-Fos expression in this region (Uslaner et al., 2003). Future experiments designed to probe how drug-induced changes in gene expression within the OFC affect the functioning of downstream areas like the STN are warranted. The OFC also sends a minor projection to the ventral tegmental area (Geisler et al., 2007), a region known to be critically involved in the development of locomotor sensitization. It is possible that over-expression of ΔFosB in the OFC may therefore influence locomotor sensitization through this pathway.

The exact nature of the relationship between drug-induced changes in cognitive function and locomotor sensitization is currently unclear, and we have so far focused on the OFC. Given these findings, it is possible that changes in gene expression associated with the development of locomotor sensitization in other brain regions may conversely have some impact on the cognitive response to cocaine. Experiments which explore the interplay between cortical and subcortical areas following administration of addictive drugs may shed new light on how the addicted state is generated and maintained, and the interactive roles played by sensitization and tolerance in this process.

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