Striatal Cell Type-Specific Overexpression of ΔFosB Enhances Incentive for Cocaine

Christina R. Colby,[1] Kim Whisler,[2] Cathy Steffen,[2] Eric J. Nestler,[2] and David W. Self[2]

[1]Division of Molecular Psychiatry, Yale University School of Medicine and Connecticut Mental Health Center, New Haven, Connecticut 06508, and [2]Department of Psychiatry, The Seay Center for Basic and Applied Research in Psychiatric Illness, The University of Texas Southwestern Medical Center, Dallas, Texas 75390-9070

The transcription factor ΔFosB accumulates in substance P– dynorphin-containing striatal neurons with repeated cocaine use. Here, we show that inducible transgenic ΔFosB overexpression in this same striatal cell type facilitates acquisition of cocaine self-administration at low-threshold doses, consistent with increased sensitivity to the pharmacological effects of the drug. Importantly, ΔFosB also enhances the degree of effort mice will exert to maintain self-administration of higher doses on a progressive ratio schedule of reinforcement, whereas levels of cocaine intake are not altered on less demanding fixed-ratio schedules. Acquisition and extinction of behavior reinforced by food pellets is not altered in ΔFosB-overexpressing mice, indicating that ΔFosB does not alter the capacity to learn an instrumental response or cause response perseveration in the absence of reinforcement. These data suggest that accumulation of ΔFosB contributes to drug addiction by increasing the incentive properties of cocaine, an effect that could increase the risk for relapse long after cocaine use ceases.

Key words: **cocaine; reinforcement; reward; addiction; nucleus accumbens; craving**

Introduction

Neuroadaptations to repeated drug use are thought to underlie many addiction-related changes in behavior (Self and Nestler, 1998; Koob and Le Moal, 2001; Nestler, 2001). One such neuroadaptation involves accumulation of the transcription factor ΔFosB, a highly stable product of the *fosB* gene (Hope et al., 1994; Chen et al., 1997). Unlike other Fos-related proteins induced by acute drug treatment, ΔFosB accumulates only in striatal neurons after repeated exposure to cocaine and other drugs of abuse (Nye et al., 1995; Nye and Nestler, 1996; Chen et al., 1997; Pich et al., 1997), primarily because of its slow rate of degradation (Chen et al., 1997). ΔFosB is induced selectively in the substance P–dynorphin-containing neurons of the striatum (Nye et al., 1995; Moratalla et al., 1996), which coexpress primarily D_1 dopamine receptors and project to midbrain structures, including the ventral tegmental area (Lu et al., 1998; Steiner and Gerfen, 1998; Aubert et al., 2000; Canales and Graybiel, 2000). Drug-induced accumulation of ΔFosB in nucleus accumbens, a ventral striatal brain region highly implicated in regulation of motivated behaviors, suggests that ΔFosB could contribute to certain transitional changes underlying the addiction process, such as escalating drug intake and increased drug craving.

Using a tetracycline-inducible, cell-specific transgenic system, we found previously that ΔFosB overexpression in substance P–dynorphin-containing striatal neurons increases sensitivity to the pharmacological properties of low doses of cocaine, suggesting that ΔFosB accumulation contributes to pharmacological sensitization (Kelz et al., 1999). However, the addicted phenotype modeled in self-administration studies is not related directly to leftward shifts in dose sensitivity but rather to vertical shifts in dose-intake function and to enhanced motivation to seek cocaine in the absence of reinforcement (Ahmed and Koob, 1998; Mendrek et al., 1998; Lorrain et al., 2000; Piazza et al., 2000; Sutton et al., 2000). The latter reflects sensitization to the incentive properties of cocaine, an effect thought to underlie craving and relapse during withdrawal (Robinson and Berridge, 2001). In this study, we tested whether accumulation of ΔFosB in striatum contributes to such addiction-related changes in cocaine self-administration using procedures designed to measure discrete aspects of cocaine-taking and -seeking behaviors.

Materials and Methods

Mice. Male bigenic mice were derived from a cross between transgenic homozygotes with neuron-specific enolase (NSE)-tTA (tetracycline transactivator protein) (line A) and tetOp (tetracycline-responsive promoter)-Δ*fosB* (line 11d), with both parental lines maintained on a mixed outbred background (50% ICR, 50% C57BL/6 × SJL) as described previously (Kelz et al., 1999). All mice were conceived and raised on 100 μg/ml doxycycline (Sigma, St. Louis, MO) in the drinking water. At weaning, mice destined for the ΔFosB group were switched to water for 8–11 weeks before behavioral testing commenced, whereas controls remained on doxycycline. Mice weighing 25–40 gm were housed individually in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. A serial testing procedure was implemented involving acquisition–extinction of food pellet self-administration, surgical catheter implantation, acquisition of cocaine self-administration, and self-administration dose–response testing on both fixed and progressive ratio schedules of cocaine reinforcement.

Acquisition–extinction of food pellet self-administration. Mice were food deprived for 16 hr and placed in operant test chambers (Med Associates, St. Albans, VT) for an initial 1 hr period to measure spontaneous lever press behavior in the absence of reinforcement. On subsequent test

Received Oct. 31, 2002; revised Dec. 26, 2002; accepted Dec. 31, 2002.

This work was supported by United States Public Health Service Grants DA-10460 and DA-08227 and by the Lydia Bryant Test Professorship (University of Texas Southwestern Medical Center).

Correspondence should be addressed to David W. Self, Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX 75390-9070. E-mail: david.self@utsouthwestern.edu.

 0270-6474/03/232488-06$15.00/0

days, mice were allowed to lever press for food pellets consisting of standard laboratory chow (25 mg) by performing three responses at one lever (FR3) designated as active and signaled by a cue light above the lever. After each test session, mice were allowed to consume enough chow to maintain initial body weight but were food deprived for 16 hr before the subsequent test. Testing continued until mice achieved acquisition criteria (≥25 pellets per session) and continued lever-press training for an additional two test sessions. After the acquisition criterion was met, mice were fed *ad libitum* and were allowed to extinguish responding in daily 2 hr tests until mice extinction criteria were achieved (≤10 responses per lever at food-paired and inactive levers). Mice that failed to reach extinction criteria after 15 d were given an extinction latency of 15 test sessions and were not used in subsequent testing (3 of 12 control and 5 of 15 ΔFosB mice). Data from the first 27 mice were analyzed and showed no significant differences. All other mice were subjected to this procedure, and only mice that met extinction criteria were used in subsequent cocaine self-administration experiments.

Acquisition of cocaine self-administration. After achievement of extinction criteria, animals were surgically implanted with a chronic indwelling jugular catheter composed of SILASTIC tubing (0.012 inch inner diameter; 0.025 inch outer diameter) passing subcutaneously to exit the back through 23 gauge stainless steel tubing embedded in cranioplastic cement and secured with Marlex surgical mesh. Animals were allowed at least 4 d to recover before cocaine self-administration testing. Catheters were flushed daily with 0.05 ml of heparinized (20 IU/ml) bacteriostatic saline containing gentamycin sulfate (0.33 mg/ml); this antibiotic does not interact with tTA.

Each mouse was tested for acquisition of cocaine self-administration in daily 1 hr sessions in the same chamber used for food pellet self-administration. Catheters were connected to a 10 ml syringe pump (Razel Scientific Instruments, Stamford, CT) through a fluid swivel (Stoelting, Kiel, WI). During acquisition of cocaine self-administration, a single lever-press response on the active lever (FR1) delivered an intravenous cocaine injection in 50 μl of saline over 2.5 sec. A cue stimulus consisting of a cue light above the lever, house light off, and injection pump sound was concurrent with each injection, followed by an additional 8 sec time-out when responding had no programmed consequences. Acquisition of cocaine self-administration was conducted over 10 d, with access to a low-threshold dose for the first 5 d (125 or 250 μg/kg per injection), followed by a higher dose (500 μg/kg per injection) for days 6–10. This procedure ensured acquisition of cocaine self-administration in most mice after 10 test sessions. Catheter patency was verified after each test phase (acquisition, fixed-ratio dose–response, and progressive ratio dose–response) with sodium methohexital (0.5 mg/ml).

Cocaine self-administration dose–response on fixed and progressive ratio schedules. After acquisition testing, mice continued to self-administer cocaine at 500 μg/kg per injection in daily 1 hr sessions, and the response requirement was increased incrementally to a fixed ratio of five responses per injection (FR5) and until daily cocaine intake stabilized to within 15% of the mean of three consecutive sessions. Mice were subsequently allowed to self-administer descending injection doses of cocaine, each for two consecutive daily 1 hr sessions, beginning with 1000 μg/kg per injection and ending with saline; the number of cocaine injections per session and the total amount of cocaine intake per session from the second test were used for the analysis. Thirty-one of 41 mice entering fixed-ratio dose–response testing maintained catheter patency and were used in the analysis.

After fixed-ratio dose–response testing, animals were restabilized at 500 μg/kg per injection for at least 3 d and subsequently allowed to self-administer cocaine on a progressive ratio schedule, with the response requirement for each successive injection increasing by progressive increments according to the following series: 1, 2, 4, 6, 9, 12, 16, 20, 25, 30, 36, 42, 49, 56, 64, 72, 81, 90, 100, and 110. Three intermediate to high injection doses were chosen on the basis of their ability to maintain equivalent levels of cocaine self-administration on fixed-ratio schedules (250, 500, and 1000 μg/kg per injection). Each dose was tested in two consecutive daily sessions in counterbalanced order. The highest ratio of responses per injection achieved before a 30 min period when no additional injections were earned was analyzed for the second test day at each dose. Twenty-eight of 31 mice entering progressive ratio testing maintained catheter patency and were used in the analysis.

Data analysis. Data were analyzed by two-factor ANOVA on group with repeated measures on either dose or time. *Post hoc* comparisons between controls and ΔFosB mice used tests for simple effects at each time point or cocaine dose. Unitary measures (e.g., latency) were compared with unpaired *t* tests. Separate within-group ANOVAs were conducted on self-administration dose–response (fixed ratio), and *post hoc* analysis compared the number of injections at each cocaine dose with saline by Dunnett's test; significant effects are shown for the 63 μg/kg per injection dose only.

Results

In male bigenic mice (NSE-tTA × tetOp-Δ*fosB*), removal of doxycycline from the drinking water at weaning induces a 7.5-fold increase in ΔFosB immunoreactivity at adulthood that is restricted to substance P–dynorphin-containing striatal neurons, and no appreciable expression is seen in other brain regions (Chen et al., 1998; Kelz et al., 1999). We first tested these mice for possible differences in instrumental behavior using 25 mg food pellets as a reinforcer while they were maintained on a food-restricted diet. Figure 1*B* shows that mice overexpressing ΔFosB and their bigenic littermate controls (maintained on doxycycline) sample the levers at equivalent rates in the absence of reinforcement during an initial test for spontaneous lever-press behavior. When food reinforcement is made available on a FR3 schedule (three responses per pellet), both ΔFosB mice and controls acquire lever-press responding at similar rates (Fig. 1*A*), averaging approximately five to six test sessions to achieve acquisition criteria (first session in which ≥25 pellets are earned). Acquisition latencies range from 1 to 13 d in controls and 1 to 9 d in ΔFosB-overexpressing mice.

Both ΔFosB and control mice showed a similar preference for the active lever on achieving acquisition criteria and consumed the 30-pellet allotment in similar times after 2 additional training days (Fig. 1*B*). After food pellet self-administration, both ΔFosB and control mice extinguished responding at similar rates in the absence of reinforcement (Fig. 1*C*), requiring an average of eight sessions to achieve extinction criteria (≤10 responses in 2 hr at both levers). These data indicate that ΔFosB-over-expressing mice and doxycycline controls are equally capable of acquiring and discriminating instrumental responses and equally capable of extinction learning when reinforcement is not available.

Acquisition of cocaine self-administration was tested in daily 1 hr sessions over 10 d on a FR1 reinforcement schedule, with an 8 sec time-out period after each cocaine injection. Figure 2*A* shows that ΔFosB-overexpressing mice avidly learn to self-administer a low-threshold dose of cocaine (125 μg/kg per injection), taking significantly more cocaine injections than doxycycline controls after 4 d of acquisition testing ($F_{(1,22)} = 5.806$; $p = 0.025$). Total active lever responses increase progressively only in ΔFosB mice at this low dose during acquisition (lever × test day; $F_{(4,40)} = 2.823$; $p = 0.037$), although both ΔFosB ($F_{(1,10)} = 16.505$; $p = 0.002$) and control ($F_{(1,12)} = 6.087$; $p = 0.030$) mice respond more at the active than the inactive lever. ΔFosB mice achieve acquisition criteria for cocaine self-administration faster than doxycycline controls, as indicated by the number of test sessions required to achieve >15 cocaine injections for 3 consecutive days, each with a 3:1 ratio of active–inactive lever presses. Both groups show similar self-administration rates when the injection dose is raised to 500 μg/kg per injection over days 6–10 of acquisition testing, indicating that increased self-administration at the lower dose does not reflect a generalized rate-enhancing effect in ΔFosB mice that self-administer cocaine. Indeed, when

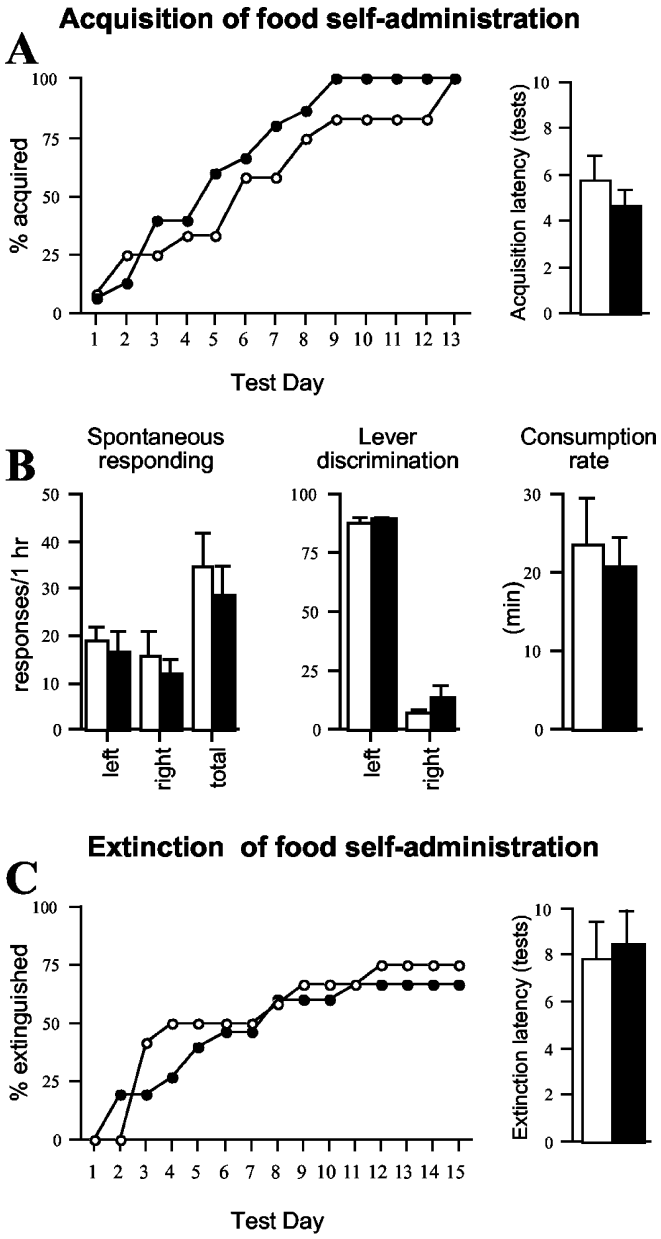

Figure 1. Striatal cell-specific overexpression of ΔFosB ($n = 15$; *filled circles* and *bars*) fails to alter acquisition and extinction of lever-press responding reinforced by food pellets compared with bigenic littermate controls maintained on doxycycline ($n = 12$; *open circles* and *bars*). *A*, The percentage of mice achieving acquisition criteria (for criteria, see Results) in daily 1 hr tests and the mean ± SEM number of test sessions to acquire self-administration of food pellets on an FR3 reinforcement schedule are shown. *B*, Spontaneous lever-press responding in naive mice before acquisition testing is similar between groups (*left*), and ΔFosB overexpression also fails to alter lever discrimination on the day acquisition criteria are met (*middle*) or the latency to consume 30 food pellets after 2 additional training days (*right*). *C*, The percentage of ΔFosB mice achieving extinction criteria (≤10 responses in 2 hr at both levers) and the number of test sessions to achieve extinction criteria in the absence of reinforcement are similar in ΔFosB and control mice.

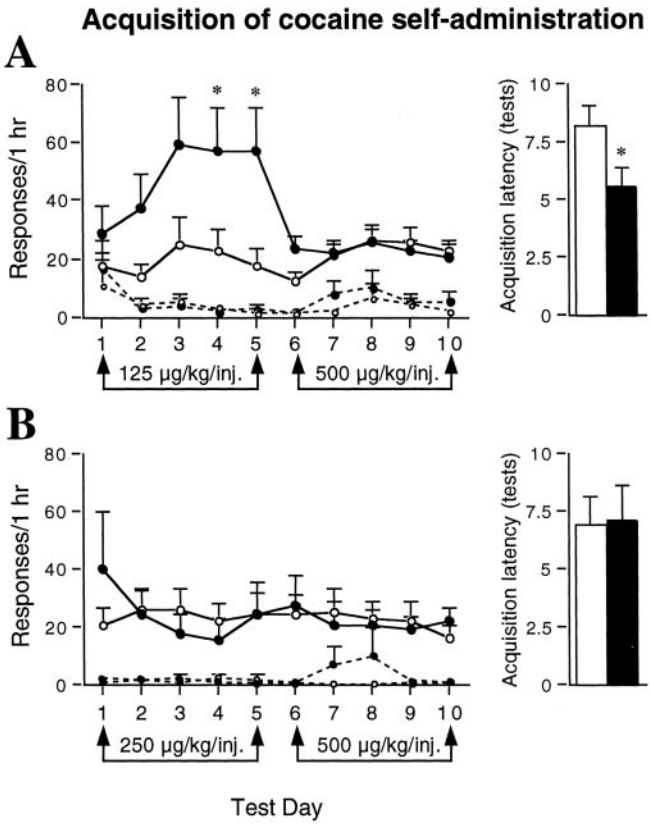

Figure 2. Striatal cell-specific overexpression of ΔFosB (*filled circles* and *bars*) facilitates acquisition of cocaine self-administration (FR1) at a low-threshold dose of cocaine (125 μg/kg per injection) (*A*) but not at a higher suprathreshold dose (250 μg/kg per injection) (*B*) compared with littermate bigenic controls maintained on doxycycline (*open circles* and *bars*). The numbers of cocaine injections (*solid lines*) and inactive lever presses (*dashed lines*) are shown at *left*, and the number of test sessions (latency) to achieve criteria for acquisition of cocaine self-administration are shown at *right* (for criteria, see Results). Each dose is tested for 5 d, followed by a higher training dose (500 μg/kg per injection) for days 6–10 to demonstrate a capacity for acquisition in all mice used in the analysis. *Asterisks* indicate that ΔFosB mice ($n = 11, 7$) differ from littermate controls ($n = 13, 10$) for threshold–dose cocaine self-administration by tests for simple effects ($p < 0.05$).

mice are allowed to initiate cocaine self-administration at a higher injection dose for the first 5 test days (250 μg/kg per injection), there is no difference in response rates or the latency to acquire self-administration (Fig. 2*B*). These data indicate that ΔFosB increases sensitivity to the reinforcing effects of very low doses of cocaine, consistent with increased sensitivity to cocaine in locomotor and place preference tests reported previously (Kelz et al., 1999). However, 7 of 25 ΔFosB mice completing acquisition testing with patent catheters (5 of 16 at 125 μg/kg; 2 of 9 at 250 μg/kg) failed to achieve acquisition criteria compared with only 1 of 24 controls (1 of 14 at 125 μg/kg), and analysis of acquisition rates is limited to animals that demonstrate a capacity to acquire cocaine self-administration. These latter differences are not statistically significant but could suggest that ΔFosB mice that fail to acquire cocaine self-administration at low doses are more refractory than controls to subsequent acquisition at higher doses.

After 10 d of acquisition testing, mice that met acquisition criteria (41 of 49) were trained to self-administer cocaine at 500 μg/kg per injection in daily 1 hr sessions, and the response requirement was gradually increased to five lever presses per injection until self-administration rates stabilized to within 15%. Mice were subsequently tested with descending injection doses, each available for 2 consecutive days over 12 d of testing; 31 of 41 mice maintained catheter patency throughout this procedure. Cocaine self-administration produces an inverted U-shaped dose–response curve on fixed-ratio schedules, spanning dose thresholds for maintaining self-administration, and a descending limb in which increasing the injection dose prolongs the duration of cocaine reward, resulting

in fewer self-injections. Figure 3*A* shows that both ΔFosB-overexpressing mice and doxycycline controls show typical inverted U-shaped self-administration dose–response curves. However, the lowest cocaine dose (63 μg/kg per injection) maintains self-administration rates above saline injections only in ΔFosB mice but not in controls, consistent with enhanced sensitivity to cocaine reinforcement at low doses in acquisition testing. At higher doses that maintain self-administration in both groups, ΔFosB fails to alter self-administration rates on the descending limb of the curve, and overall cocaine intake is similar for both groups across these injection doses (Fig. 3*B*), indicating that ΔFosB overexpression does not alter the duration of reward produced by the cocaine injections.

After dose–response testing on a fixed-ratio schedule, mice returned to the training dose and were stabilized for at least 3 d before subsequent testing on a progressive ratio schedule of cocaine reinforcement. In progressive ratio testing, each successive cocaine injection requires a progressively greater number of lever-press responses; the highest ratio of lever presses per cocaine injection achieved before self-administration ceases represents the degree of effort animals will exert to maintain cocaine self-administration and is thought to reflect the incentive strength of cocaine. Figure 4*A* shows representative cumulative response records from individual control and ΔFosB-overexpressing mice self-administering cocaine (250 μg/kg per injection) on the progressive ratio schedule. The ΔFosB mouse achieves a higher ratio of responses per injection before self-administration ceases, performing 56 responses for the final cocaine injection, as indicated by the vertical difference between the last two injections (*dotted lines*), relative to 30 responses per injection in the control mouse. ΔFosB mice complete higher response ratios at 250 and 500 μg/kg per injection ($F_{(1,26)} = 4.620$; $p = 0.041$), indicating greater effort to maintain self-administration (Fig. 4*B*). A main effect of dose indicates that the highest ratio of responses per injection completed increases in a dose-dependent manner ($F_{(2,52)} = 5.984$; $p = 0.005$), although convergence at the highest dose probably reflects a performance ceiling in both groups. Moreover, the fact that the final ratio completed converges at this high dose indicates that increased response ratios at lower doses in ΔFosB mice are not related to a generalized enhancement of performance capacity but rather to greater motivation to obtain cocaine reinforcement.

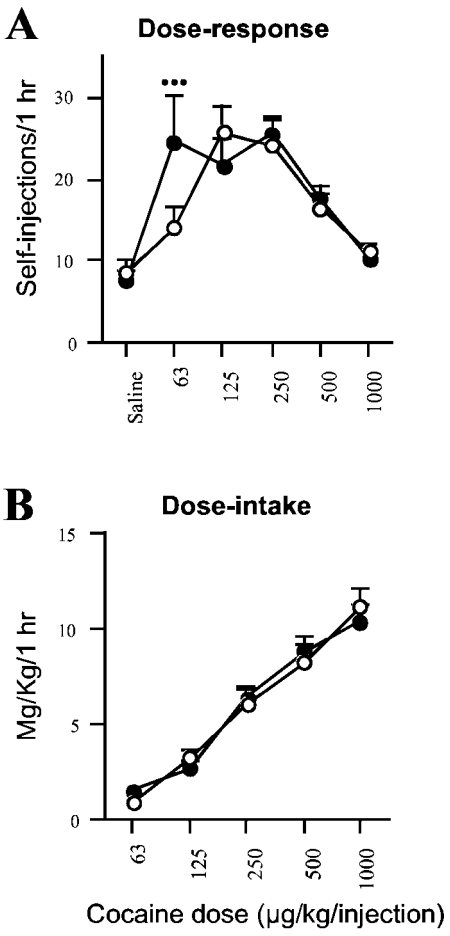

Figure 3. Striatal cell-specific overexpression of ΔFosB increases sensitivity to a low threshold dose of cocaine after acquisition and stabilization of self-administration on an FR5 schedule ($n = 16$; *filled circles*) but does not alter self-administration rates at higher doses that are reinforcing in littermate controls ($n = 17$; *open circles*) (*A*) or overall cocaine intake across all doses (*B*). *Black dots* indicate that the 63 μg/kg per injection dose differs from saline by Dunnett's test ($p < 0.001$).

Discussion

Striatal cell-specific overexpression of ΔFosB in inducible transgenic mice produces two major effects on cocaine self-administration behavior. The first enhances sensitivity to the reinforcing effects of very low injection doses of cocaine. Thus, ΔFosB-overexpressing mice will learn to self-administer cocaine at doses lower than controls and will maintain self-administration of even lower doses after cocaine self-administration is acquired. These results are entirely consistent with a role for ΔFosB in substance P–dynorphin-containing striatal neurons in regulating pharmacological sensitivity to cocaine (Kelz et al., 1999) and show that enhanced sensitivity translates into a propensity to acquire cocaine self-administration at these low doses. Such increased sensitivity to cocaine is not caused by altered pharmacokinetics by ΔFosB or doxycycline because (1) we reported previously that ΔFosB overexpression in these mice fails to alter serum cocaine levels (Kelz et al., 1999), (2) the NSE promoter restricts transgenic expression to neural tissue, and (3) doxycycline treatment fails to alter serum cocaine levels and locomotor responses to acute and repeated cocaine administration in monogenic NSE-tTA or tetOp-ΔFosB mice (data not shown). Moreover, both groups self-administer similar amounts of cocaine at higher doses. Increased acquisition at a low cocaine dose in ΔFosB mice also is unrelated to facilitation of instrumental learning or response perseveration, because both ΔFosB mice and controls acquire responding at similar rates when reinforced by higher cocaine doses or by food pellets and extinguish at similar rates in the absence of reinforcement.

Importantly, cell-specific overexpression of ΔFosB also raises the amount of effort mice will exert to maintain cocaine self-administration. Given that ΔFosB and control mice reach similar asymptotic response ratios in progressive ratio testing, these differences cannot be ascribed to a generalized performance-enhancing effect. Instead, these results suggest that ΔFosB sensitizes animals to the incentive properties of cocaine, thereby increasing the motivation to seek cocaine when reinforcement is withheld. Sensitization to the incentive properties of cocaine differs markedly from an increase in pharmacological sensitivity discussed above, because ΔFosB mice work harder to maintain self-administration of doses that also are effective reinforcers in control mice. Thus, both groups readily acquire and take similar amounts of cocaine at the 250 and 500 μg/kg injection doses when self-administered on less demanding fixed-ratio schedules of reinforcement, but ΔFosB mice work harder to self-administer these same doses on a progressive ratio schedule.

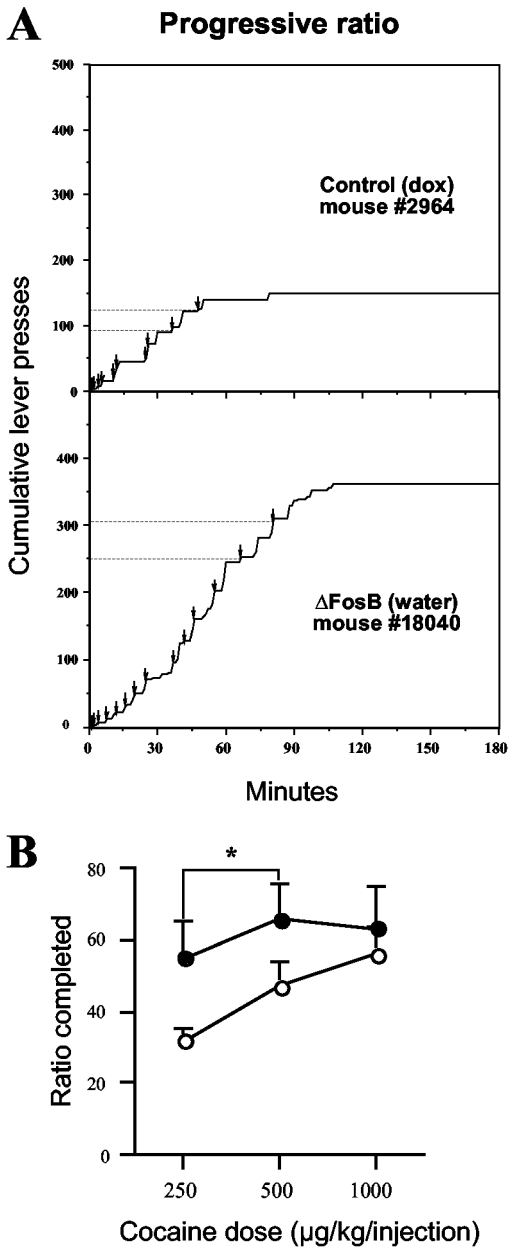

Figure 4. Striatal cell-specific overexpression of ΔFosB facilitates cocaine self-administration on a progressive ratio schedule of reinforcement. *A*, Cumulative active lever response records for representative mice show that ΔFosB increases the number of cocaine injections (*arrows*) earned relative to a bigenic littermate control (250 μg/kg per injection). *dox*, Doxycycline. *B*, ΔFosB mice ($n = 11$; *filled circles*) exert greater effort to maintain cocaine self-administration, as reflected by completing a higher ratio of responses per injection (distance between *dotted lines* in *A*) immediately before cessation of self-administration. Convergence of the final ratio completed at the highest injection dose indicates that littermate control mice ($n = 17$; *open circles*) are capable of performing at levels found in ΔFosB mice. *Asterisk* indicates main effect of group on ratio completed by ANOVA across the 250 and 500 μg/kg per injection dose ($p < 0.05$).

Sensitization to the incentive properties of cocaine can be dissociated from pharmacological sensitivity and regulation of drug intake in self-administration studies (Richardson and Roberts, 1996; Green et al., 2002), suggesting that these phenomena are independently regulated by separate neural substrates. For example, rats raised in isolated environmental conditions have lower dose thresholds for amphetamine self-administration than rats raised in enriched conditions, but they exert equal effort to maintain self-administration of suprathreshold doses (Green et al., 2002). In contrast, increased motivation for suprathreshold doses of drug is arguably more relevant to craving in humans than pharmacological sensitivity. In this regard, it is notable that attempts to model the addicted phenotype in animals, whether by inherent differences or by transitional changes in drug self-administration, have not found overall leftward shifts in dose sensitivity but instead are related to higher levels of drug intake and drug-seeking behavior (Ahmed and Koob, 1998; Mendrek et al., 1998; Deroche et al., 1999; Ahmed et al., 2000; Lorrain et al., 2000; Piazza et al., 2000; Sutton et al., 2000).

ΔFosB accumulates in both dorsal and ventral striatum with repeated cocaine exposure, and this regional pattern of expression is reproduced in the inducible ΔFosB mice used in these studies. However, the effects of ΔFosB on cocaine self-administration may result from ΔFosB accumulation in ventral striatal regions, including the nucleus accumbens, because cocaine self-administration is modulated by dopaminergic lesions of the nucleus accumbens and not the caudate–putamen (Koob and Goeders, 1989). An important advantage in our studies is that the transcription factor is expressed only in substance P–dynorphin containing striatal neurons, precisely the same cell type in which endogenous ΔFosB accumulates with repeated cocaine exposure (Nye et al., 1995; Moratalla et al., 1996). In nucleus accumbens, these neurons project primarily to the ventral tegmental area and constitute a major output pathway from the basal ganglia (Lu et al., 1998; Steiner and Gerfen, 1998; Aubert et al., 2000; Canales and Graybiel, 2000).

ΔFosB is induced by a cascade of events thought to involve cocaine-induced elevations in nucleus accumbens dopamine levels and activation of D_1 dopamine receptor–cAMP-mediated signaling pathways (Nye et al., 1995). Accumulation of ΔFosB in these neurons would be expected to alter expression of multiple genes that contain activator protein 1 (AP-1) binding sites in their promoter regions. One of these AP-1 targets is the cyclin-dependent kinase CDK-5 that is induced by both ΔFosB overexpression and chronic cocaine (Bibb et al., 2001). ΔFosB-induced expression of CDK-5 may represent a negative feedback signaling pathway, because CDK-5 converts DARPP-32 (dopamine-regulated phosphoprotein 32) into a cAMP-dependent protein kinase inhibitor. Thus, CDK-5 expression may counteract the acute effects of cocaine mediated by D_1 receptors and cAMP and upregulation of cAMP signaling proteins in nucleus accumbens produced by chronic cocaine (Terwilliger et al., 1991; Freeman et al., 2001). In another sense, ΔFosB induction of CDK-5 expression and the subsequent inhibition of cAMP signaling could contribute to enhanced motivation for cocaine by ΔFosB, because we found previously that inhibition of cAMP-dependent protein kinase in nucleus accumbens promotes cocaine-seeking behavior (Self et al., 1998), an effect entirely consistent with ΔFosB action in progressive ratio testing.

Chronic cocaine use produces numerous neuroadaptations in the brain, but very few have been shown, as ΔFosB is shown here, to cause addiction-related changes in drug self-administration. Our previous studies suggest that drug-induced upregulation of nucleus accumbens cAMP signaling could contribute to escalating cocaine intake as initial recreational use leads to cocaine addiction, consistent with an intracellular mechanism of tolerance to cocaine reward (Self et al., 1998). The fact that ΔFosB accumulation does not affect overall cocaine intake suggests that other cAMP-mediated cellular events contribute to this escalation. In contrast, striatal accumulation of ΔFosB could enhance incentive for cocaine as the addiction process advances, thereby sensitizing mech-

anisms that regulate drug craving and relapse. Moreover, these results emphasize the notion that drug-taking and -seeking behaviors are regulated by different neuronal processes and illustrate the importance of studying each drug-induced neuroadaptation in the context of discrete aspects of drug self-administration behavior.

Another consideration is that most neuroadaptations to chronic drug use, including ΔFosB, inevitably return to normal after withdrawal from self-administration. However, neuroadaptations that directly increase the incentive properties of drugs could inevitably lead to long-term, even permanent, influences by facilitating the motivational salience of drug-related memories. Thus, by directly increasing the incentive value of cocaine during drug use, ΔFosB could indirectly facilitate drug craving triggered by environmental stimuli associated with cocaine use, even after ΔFosB levels return to normal in prolonged abstinence. Similar incentive learning during early heroin withdrawal can subsequently influence the motivation to seek heroin long after withdrawal symptoms subside (Hutcheson et al., 2001) and could also outlast certain neuroadaptations to repeated drug use. Alternatively, relatively transient neuroadaptations in transcription factors like ΔFosB could instigate a cascade of neuronal events leading to relatively permanent changes in synaptic organization (Robinson et al., 2001) that sensitize subsequent neural and motivational responses to cocaine or cocaine-related environmental stimuli. Additional investigation on how ΔFosB and other neuroadaptations contribute to the long-term consequences of drug addiction ultimately will provide new opportunities to reverse or prevent neurobehavioral processes that underlie persistent addictive behavior.

References

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science 282:298–300.

Ahmed SH, Walker JR, Koob GF (2000) Persistent increase in the motivation to take heroin in rats with a history of drug escalation. Neuropsychopharmacology 22:413–421.

Aubert I, Ghorayeb I, Normand E, Bloch B (2000) Phenotypical characterization of the neurons expressing the D1 and D2 dopamine receptors in the monkey striatum. J Comp Neurol 418:22–32.

Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, Yan Z, Sagawa ZK, Ouimet CC, Nairn AC, Nestler EJ, Greengard P (2001) Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature 410:376–380.

Canales JJ, Graybiel AM (2000) Patterns of gene expression and behavior induced by chronic dopamine treatments. Ann Neurol 47:S53–S59.

Chen J-S, Kelz MB, Hope BT, Nakabeppu Y, Nestler EJ (1997) Chronic Fos-related antigens: stable variants of ΔFosB induced in brain by chronic treatments. J Neurosci 17:4933–4941.

Chen J-S, Kelz MB, Zeng G, Sakai N, Steffan C, Shockett PE, Picciotto MR, Duman RS, Nestler EJ (1998) Transgenic animals with inducible, targeted gene expression in brain. Mol Pharmacol 54:495–503.

Deroche V, Le Moal M, Piazza PV (1999) Cocaine self-administration increases the incentive motivational properties of the drug in rats. Eur J Neurosci 11:2731–2736.

Freeman WM, Nader MA, Nader SH, Robertson DJ, Gioia L, Mitchell SM, Daunais JB, Porrino LJ, Friedman DP, Vrana KE (2001) Chronic cocaine-mediated changes in non-human primate nucleus accumbens gene expression. J Neurochem 77:542–549.

Green TA, Gehrke BJ, Bardo MT (2002) Environmental enrichment decreases intravenous amphetamine self-administration in rats: dose-response functions for fixed and progressive-ratio schedules. Psychopharmacology 162:373–378.

Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS, Nestler EJ (1994) Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. Neuron 13:1235–1244.

Hutcheson DM, Everitt BJ, Robbins TW, Dickinson A (2001) The role of withdrawal in heroin addiction: enhances reward or promotes avoidance? Nat Neurosci 4:943–947.

Kelz MB, Chen JS, Carlezon Jr WA, Whisler K, Gilden L, Beckmann A, Steffan C, Zheng Y-J, Marotti L, Self DW, Tkatch T, Baranauskas G, Surmeier DJ, Neve RL, Duman RS, Picciotto MR, Nestler EJ (1999) Expression of the transcription factor ΔFosB in the brain controls sensitivity to cocaine. Nature 401:272–276.

Koob GF, Goeders NE (1989) Neuroanatomical substrates of drug self-administration. In: The neuropharmacological basis of reward (Leibman JM, Cooper SJ, eds), pp 214–263. Oxford: Clarendon.

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97–129.

Lorrain DS, Arnold GM, Vezina P (2000) Previous exposure to amphetamine increases incentive to obtain the drug: long-lasting effects revealed by the progressive ratio schedule. Behav Brain Res 107:9–19.

Lu XY, Ghasemzadeh MB, Kalivas PW (1998) Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. Neuroscience 82:767–780.

Mendrek A, Blaha CD, Phillips AG (1998) Pre-exposure of rats to amphetamine sensitizes self-administration of this drug under a progressive ratio schedule. Psychopharmacology 135:416–422.

Moratalla R, Elibol B, Vallejo M, Graybiel AM (1996) Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. Neuron 17:147–156.

Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2:119–128.

Nye HE, Nestler EJ (1996) Induction of chronic Fos-related antigens in rat brain by chronic morphine administration. Mol Pharmacol 49:636–645.

Nye HE, Hope BT, Kelz MB, Iadarola M, Nestler EJ (1995) Pharmacological studies of the regulation of chronic FOS-related antigen induction by cocaine in the striatum and nucleus accumbens. J Pharmacol Exp Ther 275:1671–1680.

Piazza PV, Deroche-Gamonent V, Rouge-Pont F, Le Moal M (2000) Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. J Neurosci 20:4226–4232.

Pich EM, Pagliusi SR, Tessari M, Talabot-Ayer D, Hooft van Huijsduijnen R, Chiamulera C (1997) Common neural substrates for the addictive properties of nicotine and cocaine. Science 275:83–86.

Richardson NR, Roberts DC (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods 66:1–11.

Robinson TE, Berridge KC (2001) Incentive-sensitization and addiction. Addiction 96:103–114.

Robinson TE, Gorny G, Mitton E, Kolb B (2001) Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. Synapse 39:257–266.

Self DW, Nestler EJ (1998) Relapse to drug seeking: neural and molecular mechanisms. Drug Alcohol Depend 51:49–60.

Self DW, Genova LM, Hope BT, Barnhart WJ, Spencer JJ, Nestler EJ (1998) Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. J Neurosci 18:1848–1859.

Steiner H, Gerfen CR (1998) Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. Exp Brain Res 123:60–76.

Sutton MA, Karanian DA, Self DW (2000) Factors that determine a propensity for cocaine-seeking behavior during abstinence in rats. Neuropsychopharmacology 22:626–641.

Terwilliger RZ, Beitner-Johnson D, Sevarino KA, Crain SM, Nestler EJ (1991) A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. Brain Res 548:100–110.