Published in final edited form as:
Biol Psychiatry. 2012 January 1; 71(1): 44–50. doi:10.1016/j.biopsych.2011.08.011.

ΔFosB enhances the rewarding effects of cocaine while reducing the pro-depressive effects of the kappa-opioid agonist U50488

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Abstract

Background—Elevated expression of the transcription factor ΔFosB accompanies repeated exposure to drugs of abuse, particularly in brain areas associated with reward and motivation (e.g., nucleus accumbens [NAc]). The persistent effects of ΔFosB on target genes may play an important role in the development and expression of behavioral adaptations that characterize addiction. This study examines how ΔFosB influences the responsiveness of the brain reward system to rewarding and aversive drugs.

Methods—We used the intracranial self-stimulation (ICSS) paradigm to assess the effects of cocaine in transgenic mice with inducible overexpression of ΔFosB in striatal regions (including NAc and dorsal striatum). Mice implanted with lateral hypothalamic stimulating electrodes were trained using the 'rate-frequency' procedure for ICSS to determine the frequency at which stimulation becomes rewarding (threshold).

Results—A dose-effect analysis of cocaine effects revealed that mice over-expressing ΔFosB show increased sensitivity to the rewarding (threshold-lowering) effects of the drug, compared to littermate controls. Interestingly, mice over-expressing ΔFosB were also less sensitive to the pro-depressive (threshold-elevating) effects of U50488, a kappa-opioid agonist known to induce dysphoria and stress-like effects in rodents.

Conclusions—These data suggest that induction of ΔFosB in striatal regions has two important behavioral consequences—increased sensitivity to drug reward and reduced sensitivity to aversion—producing a complex phenotype that shows signs of vulnerability to addiction as well as resilience to stress.

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DISCLOSURE / CONFLICTS OF INTEREST

Over the past 3 years, Dr. Carlezon has received compensation from HUYA Biosciences and Myneurolab.com. He holds several patents and patent applications that are not related to the work described in this report. There are no personal financial holdings that may be perceived as constituting a conflict of interest. Dr. Nestler is a consultant for PsychoGenics and Merck Research Laboratories. Dr. Muschamp, Dr. Robison, and Ms. Nemeth report no biomedical financial interst or potential conflicts of interest.

Keywords

transcription factor; nucleus accumbens; brain stimulation reward; addiction; resilience; stress; model; mouse

INTRODUCTION

Exposure to drugs of abuse induces the expression of *fos* family transcription factors in neurons of the nucleus accumbens (NAc; 1), a structure implicated in drug-seeking and other motivated behaviors (2–5). While most Fos-family proteins are expressed transiently following drug exposure and this effect is attenuated with chronic dosing, ΔFosB, a splice variant of the **fosB** gene, is resistant to degradation and accumulates with repeated drug exposure (6, 7). There is now considerable evidence that persistent elevations in expression of ΔFosB within dynorphin/substance P-positive medium spiny neurons of the NAc is a neuroadaptation that leads to increased sensitivity to drugs of abuse and vulnerability to develop behaviors characteristic of addiction (8, 9). Indeed, cocaine establishes conditioned place preferences at lower doses in transgenic mice with inducible, cell-specific overexpression of ΔFosB in these neurons than in control mice (10). Additionally, ΔFosB-overexpressing mice acquire intravenous cocaine self-administration at lower doses and expend greater effort (i.e. show higher ‘breakpoints’) for cocaine infusions in progressive ratio schedules of reinforcement (11). Together, these data indicate that elevated ΔFosB in the NAc increases sensitivity to the rewarding effects of cocaine.

Several forms of chronic stress, including repeated physical restraint stress or social defeat stress, also induce ΔFosB in the NAc and several other brain regions (12–14). Such induction is seen roughly equally in dynorphin/substance P- and enkephalin-expressing medium spiny neurons. Because higher levels of ΔFosB in NAc also enhance sensitivity to natural rewards (15–17), these data may reflect a compensatory response that can potentially offset some of the aversive (dysphoric) effects of chronic stress. This possibility is supported by experiments in which wildtype mice subjected to chronic social defeat stress show a strong negative correlation between ΔFosB levels in NAc and the degree to which the mice show deleterious behavioral responses to the stress. These data are complemented by experiments in which the same line of ΔFosB-overexpressing mice that show heightened responsiveness to cocaine also show less susceptibility to chronic social defeat stress (14). As such, enhanced expression of ΔFosB in the NAc appears to engender resistance to stress (‘resilience’).

There is accumulating evidence that brain kappa-opioid receptor (KOR) systems play an important role in the motivational aspects of stress. Administration of KOR agonists produces dysphoria in humans (18, 19) and a wide variety of depressive-like effects in rodents (20–24). Importantly, KOR agonists can mimic certain aspects of stress (25–28). One mechanism by which this might occur is via interactions between the stress peptide corticotropin releasing factor (CRF) and dynorphin, the endogenous ligand for KORs (29): the aversive effects of stress appear due to CRF receptor-mediated stimulation of dynorphin release and subsequent stimulation of KORs (30, 31). In support of this mechanism, KOR antagonists block the effects of stress (20, 25, 32–35). Collectively, these findings suggest that studies of KOR agonists can provide considerable insight on the brain mechanisms of stress responsiveness in rodents.

The present studies were designed to more thoroughly evaluate how elevated expression of ΔFosB affects sensitivity to rewarding and aversive stimuli by using a single behavioral assay that is highly sensitive to both: the intracranial self-stimulation (ICSS) paradigm. In

this test, mice self-administer rewarding electrical stimulation via electrodes implanted in the lateral hypothalamus. Drugs of abuse decrease amounts of stimulation that sustain responding ("thresholds"), whereas treatments that produce anhedonia or dysphoria in people (e.g., drug withdrawal, antipsychotic agents, anti-manic agents, kappa-opioid receptor [KOR] agonists, stress) elevate ICSS thresholds, indicating that amounts of stimulation that previously sustained responding are no longer effective as the result of treatment (for review, see 36). As such, ICSS is sensitive to manipulations that increase reward, decrease reward, or increase aversion. Use of a single behavioral assay to evaluate sensitivity to rewarding and aversive stimuli is particularly advantageous in transgenic mice because it enables a standardized set of testing conditions and parameters, reducing between-assay variability in response requirements and treatment history that can complicate data interpretation. We found that mice with elevated expression of ΔFosB in dynorphin/substance P-expressing medium spiny neurons of NAc and dorsal striatum have elevated sensitivity to the rewarding effects of cocaine accompanied by decreased sensitivity to the stress-like (aversive) effects of the KOR agonist U50488, producing a phenotype that shows hallmarks of elevated vulnerability to addiction but increased resilience to stress.

MATERIALS AND METHODS

Animals

A total of 23 inducible, bitransgenic male mice expressing ΔFosB (11A line) were generated using a tetracycline-regulated gene expression system (37). Male mice carrying the NSE-tTA and TetOP-ΔFosB transgenes were raised on water containing doxycycline (DOX, 100 μg/ml; Sigma, St. Louis MO). Experiments began eight weeks after removing 13 mice from DOX to permit a stable 7-fold increase in TetOp-mediated ΔFosB transgene expression within dynorphin-positive neurons of the striatum (ΔFosB-ON; see 10, 37, 38). Eleven mice remained on DOX for the duration of experiments and constituted a control group (Control). The mice were littermates that had been backcrossed to a C57BL/6 background for at least 12 generations, and were housed individually with **ad libitum** access to food and water on a 12 h light (7:00 A.M. to 7:00 P.M.) cycle. In addition, 9 mice carrying the NSE-tTA transgene only were used as a second control group; they were raised on DOX, then removed from DOX for ~8 weeks prior to further experimentation (OFF-DOX). Procedures were conducted in accordance with the 1996 National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* and with the approval of the Institutional Animal Care and Use Committee at McLean Hospital.

Immunohistochemistry

Transgene overexpression was confirmed by immunuohistochemistry for FosB (Fig. 1). Bitransgenic mice were sacrificed and perfused trascardially with 0.1 M phosphate-buffered saline and 4% paraformaldehyde. Brains were then removed, postfixed, and cryoprotected as described previously (14, 38). Tissue was sliced on the coronal plane into 30 mm sections, and sections immunostained using a FosB antibody (SC-48, Santa Cruz Biotechnology, Santa Cruz, CA). Diaminobenzidine staining was used to visualize FosB positive cells. Images were acquired using an Zeiss Imager 1 image contrast microscope and digitally captured using Axiovison software (Carl Zeiss USA, Peabody, MA).

ICSS

Mice (25–28 g) were anesthetized with an intraperitoneal (IP) injection of a ketamine-xylazine mixture (80-10 mg/kg; Sigma) and implanted with monopolar stimulating electrodes directed stereotactically to the medial forebrain bundle (MFB; in mm from bregma, AP: −1.9, ML: −0.8, DV: −4.8 below dura, according to the atlas of Paxinos and Franklin, 2nd ed., 2001). After a one week recovery period, mice were trained to respond for

brain stimulation during daily one hour sessions (39). Stimulation current was adjusted to the lowest value that would support stable responding (60 ± 6 responses/min) for 3 consecutive days. This value was considered the "minimal current," and this approach has been used previously to identify mutation-induced differences in basal sensitivity to the rewarding effects of stimulation (40). After minimal current was measured for each mouse, it was held constant. Mice were then allowed to respond to one of 15 stimulation frequencies presented in descending order (0.05 $\log_{10}$ unit steps) during fifteen 50 sec trials. Trials were preceded by a 5 second prime where non-contingent stimulation was given, followed by a 5 sec time-out in which responding is not reinforced. Each set of 15 trials (or "pass') was presented, and responding during each 50 sec trial recorded. Over the 3–4 week course of training, the range of frequencies used was adjusted so that mice responded through the highest 6–7 frequencies stably over 6 passes (90 min of training). The lowest frequency that supported responding (ICSS threshold, or 'theta-zero') was computed using least-squares line of best fit analysis (36, 41). When animals were observed to have stable mean ICSS thresholds (±10% over 5 consecutive days), the effect of drug treatments on ICSS threshold was measured.

Drug testing

Cocaine HCl and (±)-*trans*-U50488 methanesulfanoate (Sigma) were dissolved in 0.9% saline and injected IP in a volume of 10 ml/kg. Mice responded through 3 passes immediately before drug treatment and thresholds from the second and third pass averaged to obtain the baseline (threshold and maximal response rate) parameters. Each mouse then received an injection of drug or vehicle and was tested for 15 min immediately following injections. Bitransgenic mice were given doses of cocaine (0.625–10 mg/kg) or U50488 (0.03–5.5 mg/kg) in ascending order. The OFF-DOX mice received cocaine only. Each drug treatment followed a test with vehicle on the preceding day to ensure that the mouse had recovered from prior treatments and to minimize conditioned drug effects. A two week interval was given between cocaine and U50488 experiments. As above, animals that failed to show stable baseline responding were excluded. Group differences were analyzed using *t*-test (minimum current measure), ANOVAs (effects of drug treatments on threshold and maximum rate); significant effects were analyzed further using *post hoc* tests (Dunnett's test). In each case, comparisons were made based on the null hypothesis that means in drug-treated conditions would not differ from the mean in the vehicle-treated condition. Because cocaine is known to lower reward thresholds in ICSS (42), comparisons to vehicle were made based on the hypothesis that cocaine would lower reward thresholds. Conversely, because kappa agonists have been shown to elevate reward thresholds in ICSS (23), comparisons were made to vehicle based on the hypothesis that U50488 would similarly elevate reward thresholds. Electrode placements were confirmed by histology (Fig. 2).

RESULTS

ΔFosB overexpression and minimum current measures

All mice rapidly acquired ICSS behavior and responded at high rates for MFB stimulation. There were no group differences in minimum threshold between mice overexpressing ΔFosB in striatum and NAc (ΔFosB-ON) and those maintained on DOX (Control; $t_{(22)}$=0.26, not significant [n.s.]) (Fig. 3) This indicates that the genetic manipulation itself has no effect on sensitivity to the rewarding impact of lateral hypothalamic stimulation under baseline conditions.

ΔFosB overexpression and cocaine effects

Cocaine decreased mean ICSS thresholds in all groups of mice, causing leftward shifts in ICSS rate-frequency functions (Fig. 4A,B). ΔFosB-ON mice were more sensitive to the

rewarding effects of cocaine: a 2-way repeated-measures ANOVA on mean ICSS thresholds revealed main effects of cocaine dose ($F_{(5,65)}$=11.20, P<0.01), and DOX treatment ($F_{(1,13)}$=6.23, P<0.05), but no dose×DOX interaction ($F_{(5,65)}$=0.87, n.s.). Pre-planned contrasts (Dunnett's tests) with saline vehicle treatment within each group revealed that ΔFosB-ON mice (n=8) showed significant reductions in ICSS threshold at doses ≥1.25 mg/kg, whereas a dose of 10 mg/kg was required to produce significant effects in Control (ON-DOX) mice (Fig. 4C). A 2-way repeated measures ANOVA on maximum response rates revealed a significant main effect of cocaine dose ($F_{(5,65)}$=3.89, P<0.05). Pre-planned contrasts with saline vehicle treatment within each group revealed that cocaine produced rate-increasing effects at doses ≥5 mg/kg in ΔFosB-ON mice, with no effect at any dose in Control mice (Fig. 4D). There was no main effect of DOX treatment ($F_{(1,13)}$=1.56, n.s.), nor was there a dose×DOX interaction ($F_{(5,65)}$=0.43, n.s.). DOX treatment alone had no effect on responding to the dose of cocaine tested (10 mg/kg) as Control and OFF-DOX groups showed no difference in reward thresholds (Fig. 4C, inset; $t_{(14)}$=0.27, n.s.), or maximum rates of responding (Fig. 4D, inset; $t_{(14)}$=0.34, n.s.).

ΔFosB overexpression and U50488 effects

The KOR agonist U50488 increased mean ICSS thresholds in Control mice, causing rightward shifts in the rate-frequency function of this group, whereas ΔFosB-ON mice were insensitive to the drug (Fig. 5A,B). A 2-way repeated-measures ANOVA on mean ICSS thresholds demonstrated main effects of drug dose ($F_{(6,60)}$=3.45, P<0.01), DOX treatment ($F_{(1,10)}$=18.73, P<0.01), and a significant dose×DOX interaction ($F_{(6,60)}$=2.95, P<0.05). *Post hoc* testing (Dunnett's test) showed that, compared to saline vehicle, U50488 (5.5 mg/kg) produced significant elevations of ICSS thresholds in Control mice (n=4) but had no effect in ΔFosB-ON mice (Fig. 5C). In addition, there was a significant difference between groups at this dose. A 2-way repeated-measures ANOVA on maximum response rates revealed no main effects of dose ($F_{(6,60)}$=1.95, n.s) or DOX treatment ($F_{(1,10)}$=4.66, n.s. [P=0.06]), nor was there a dose×DOX interaction ($F_{(6,60)}$=1.31, n.s.) (Fig. 5D). These data indicate that U50488 did not significantly affect responding under the conditions tested.

DISCUSSION

We show that mice with inducible overpression of ΔFosB in NAc and other striatal regions are more sensitive to the rewarding effects of cocaine and less sensitive to the prodepressive effects of the KOR agonist U50488 compared to normal mice. These data are consistent with the existing literature on the role of ΔFosB in drug reward and stress, and extend it in several important ways. Previous work with the effects of ΔFosB overexpression on drug reward used place conditioning or drug self-administration paradigms (10, 11). Data from ICSS experiments complement this work by providing a 'real-time' index of the influence of drugs on the sensitivity of brain reward circuitry. Studies in wild-type mice have shown that pharmacological manipulations can increase (e.g., cocaine) or decrease (e.g., U50488) the rewarding impact of MFB stimulation (24); ICSS thus provides a method for quantifying hedonic state while an animal is under the influence of a drug treatment. Because drugs that are known to be rewarding or aversive in humans produce opposite (i.e. lower and higher thresholds, respectively) outputs in rodent ICSS, the paradigm can more reliably dissociate these states than can drug self-administration, where lower self-administration rates could indicate either satiety or the emergence of aversive effects (36). Additionally, ICSS avoids the potential confounds that drug treatments may exert on the development and expression of learned responses in classical conditioning paradigms that are often used to study drug reward (i.e., place conditioning).

Our ICSS threshold data clearly indicate that induction of ΔFosB enhances the rewarding effects of cocaine, since the drug produces significant reductions in ICSS thresholds at lower

doses than in littermate controls in which overexpression had not been induced. The fact that the ΔFosB-ON mice also showed increases in maximum rates of responding at high doses of cocaine raises the possibility that the effect of ΔFosB overexpression on ICSS thresholds is an artifact of elevated locomotor activity or response capabilities (43). This is unlikely for several reasons. First, our method of analysis for measuring theta-0 uses a least squares line of best fit to estimate the frequency at which stimulation becomes rewarding. Because the regression algorithm discounts extreme values, it is minimally sensitive to treatment-induced alterations in response capabilities; in contrast, alterations in response capabilities alone can cause artifactual shifts in thresholds when using M-50, a measure that is analogous to an ED-50 in pharmacology (see 36, 41, 44, 45). Second, the increases in maximum response rates above baseline values are evident only at the highest doses of cocaine, two-fold higher than those at which ICSS thresholds of ΔFosB-ON animals are significantly lower than in controls. Finally, if the effects of ΔFosB on ICSS thresholds were due to non-specific activating effects of the mutation, the mice might also be expected to show greater sensitivity to the effects of MFB stimulation itself, manifested as a lower mean minimum current to support rates of 60 ± 6 responses/min, or by increases in baseline maximum response rates following treatment with vehicle. We found no evidence of either of these effects. Together, these findings suggest that ΔFosB overexpression causes elevated sensitivity to both the rewarding (at low-to-high doses) and stimulant (at high doses only) effects of cocaine. A similar pattern of effects has been previously reported in mice with a mutation that produces mania-like signs (40).

Interestingly, ΔFosB overexpression abolished the threshold-elevating, pro-depressive effects of U50488. Inasmuch as KOR agonist treatment can mimic certain effects of stress (25–28), this finding is a putative sign of resilience; indeed, ΔFosB overexpression has been associated with resilience to the depressive-like effects of chronic social defeat stress on sucrose preference and social interaction (14, 46). Stress elevates expression of dynorphin (47, 48), and KOR antagonists produce antidepressant-like and anti stress-like effects (20, 32, 47, 49). Moreover, the aversive component of hypothalamic-pituitary-adrenal axis activation that accompanies stress is mediated by dynorphin, as conditioned aversion to cues associated with swim stress or corticotrophin releasing factor are blocked by KOR antagonists or dynorphin gene knockout (30). The mice used in these experiments show selective ΔFosB overexpression in dynorphin neurons of the striatum. This in turn reduces dynorphin expression in these neurons (38), an effect that can be anticipated to reduce the baseline function of brain KOR systems. Additionally, because KOR activation attenuates the release of dopamine (DA; 22, 50), a transmitter known to play an integral role in supporting ICSS (51–53), this effect may also explain in part why ΔFosB overexpressing mice show increased sensitivity to cocaine reward. The fact that these mice have attenuated dynorphin tone together with an insensitivity to the prodepressive-like effects of exogenous KOR agonists raises the possibility that the mutation produces a more broad set of neuroadaptatons that are able to offset ‘anti-reward’ systems in the brain (54).

Regardless of whether induced by chronic exposure to drugs of abuse or by stress, the induction of ΔFosB and of dynorphin can be viewed as opposing neuroadaptations. ΔFosB appears to positively influence sensitivity to a variety of pharmacological and natural rewards (10, 11, 15). The dynorphin-KOR system, however, appears to induce prodepressive-like states that involve elements of anhedonia, dysphoria, and aversion in humans and laboratory animals (19, 21, 35, 55). Under nonpathological conditions, these adaptations may offset one another, resulting in a homeostatic-like response that compensates for external influences on hedonic tone. In light of evidence that the excitability of NAc medium spiny neurons varies inversely with mood state (14, 56, 57), ΔFosB may exert protective effects against dysphoria-inducing stressors by reducing the excitability of these cells via enhanced expression of GluR2 (10), which favors the

formation of GluR2-containing, calcium-impermeable AMPA receptors (reviewed in 58). In contrast, dynorphin or KOR agonists may attenuate the elevated levels of DA that accompany exposure to drugs of abuse (59). Addiction and depression in humans are frequently comorbid and precipitated by life stress (60–62). In contrast, the phenotype of ΔFosB overexpressing mice is one of increased drug seeking but resilience to the depressive effects of stress. The mechanisms underlying this dissociation are unclear, but it may be due to the restricted pattern of ΔFosB overexpression displayed by these mice. Elevated striatal ΔFosB and subsequent decreases in dynorphin are only two of the numerous neuroadaptations that accompany drug exposure and stress (63, 64). As such, they are unlikely to reproduce fully the set of changes that result in comorbid symptoms of addiction and depression. It is also important to emphasize that these studies address the effects of ΔFosB only, and that under normal circumstances exposure to drugs of abuse and stress cause more transient increases in the expression of other Fos family proteins not studied here, including full-length FosB (9).

In summary, we used ICSS in transgenic mice overexpressing ΔFosB to show that this genetic manipulation enhances the rewarding effects of cocaine. We also found that this confers resistance to the prodepressive effects of KOR activation by U50588. Because the dynorphin-KOR system is a key mediator of the affective consequences of stress, these data are consistent with the hypothesis that ΔFosB enhances reward sensitivity while simultaneously reducing responsiveness to stressors. As such, enhancing ΔFosB expression may under some circumstances promote resilience.

Acknowledgments

This study was supported by the National Institute on Drug Abuse and National Institute of Mental Health (DA026250 to JWM, MH51399 and DA008227 to EJN, and MH063266 to WAC).

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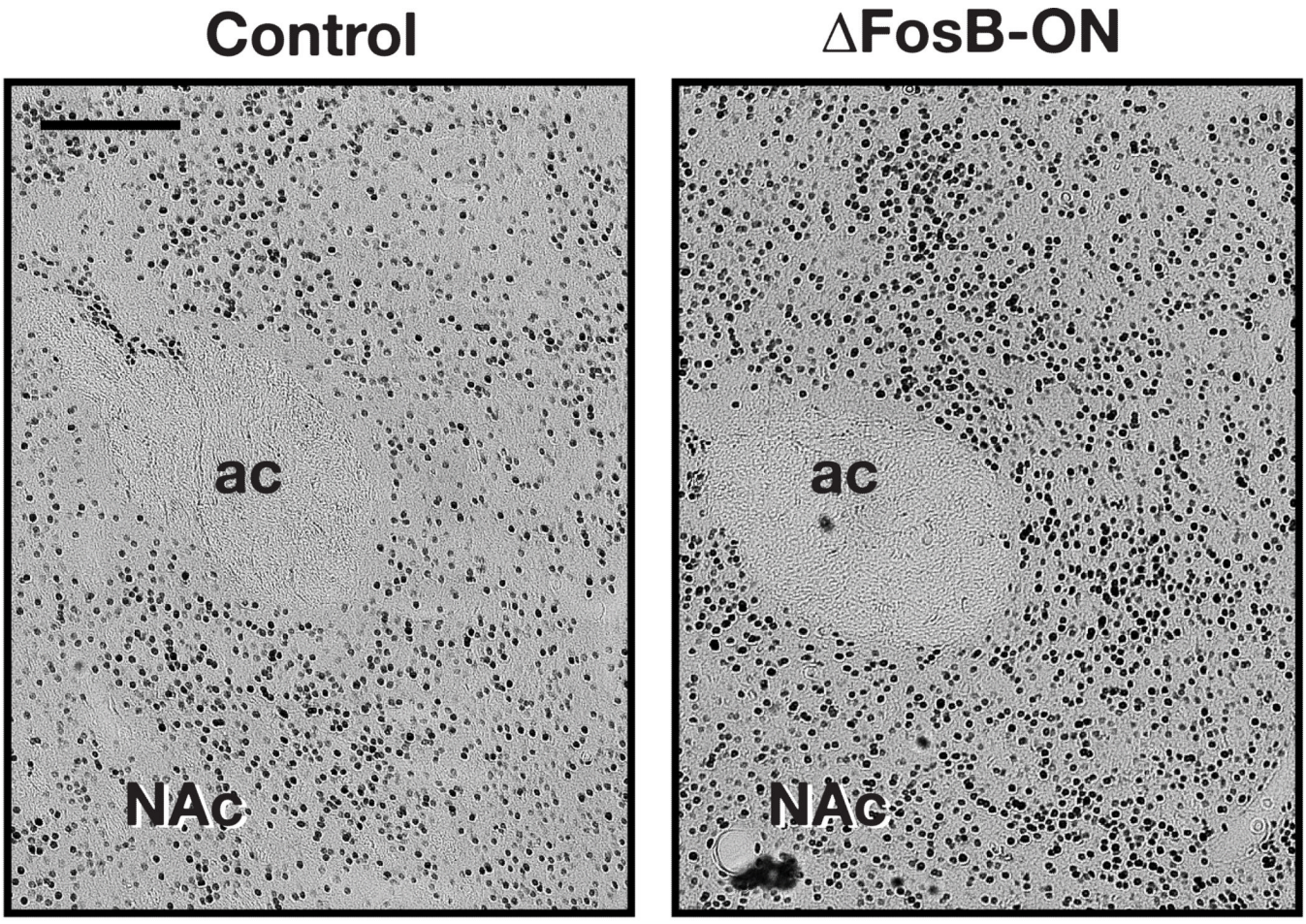

Figure 1.
Representative micrographs from bitransgenic mice showing overexpression of ΔFosB. Nuclear labeling for FosB is lower in control mice maintained on doxycycline (left panel) than those not given doxycycline (right). ac = anterior commisure; NAc = nucleus accumbens. Scale bar = 200 μm.

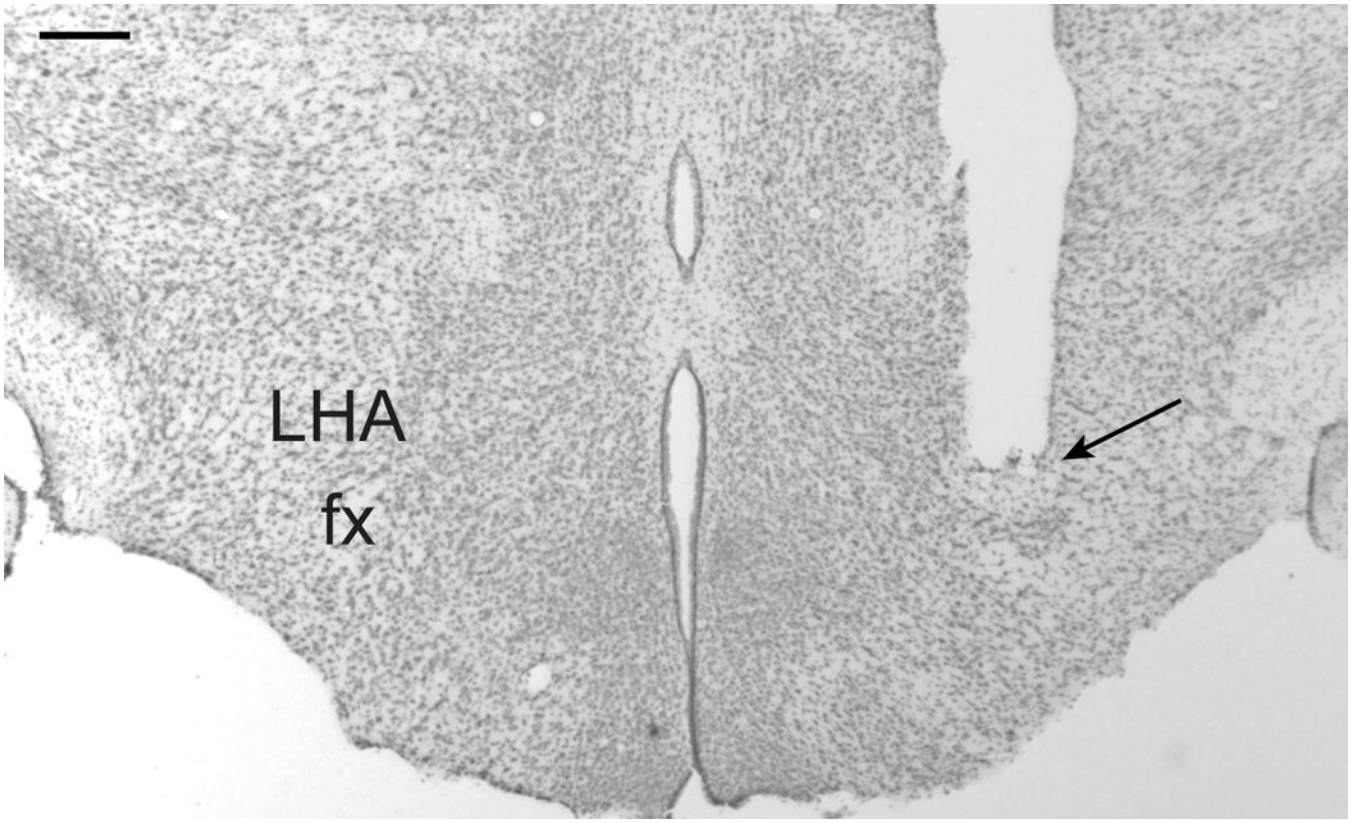

Figure 2.
Representative micrograph depicts stimulating electrode placement for ICSS (arrow). LHA = lateral hypothalamic area; fx = fornix. Scale bar = 250 μm.

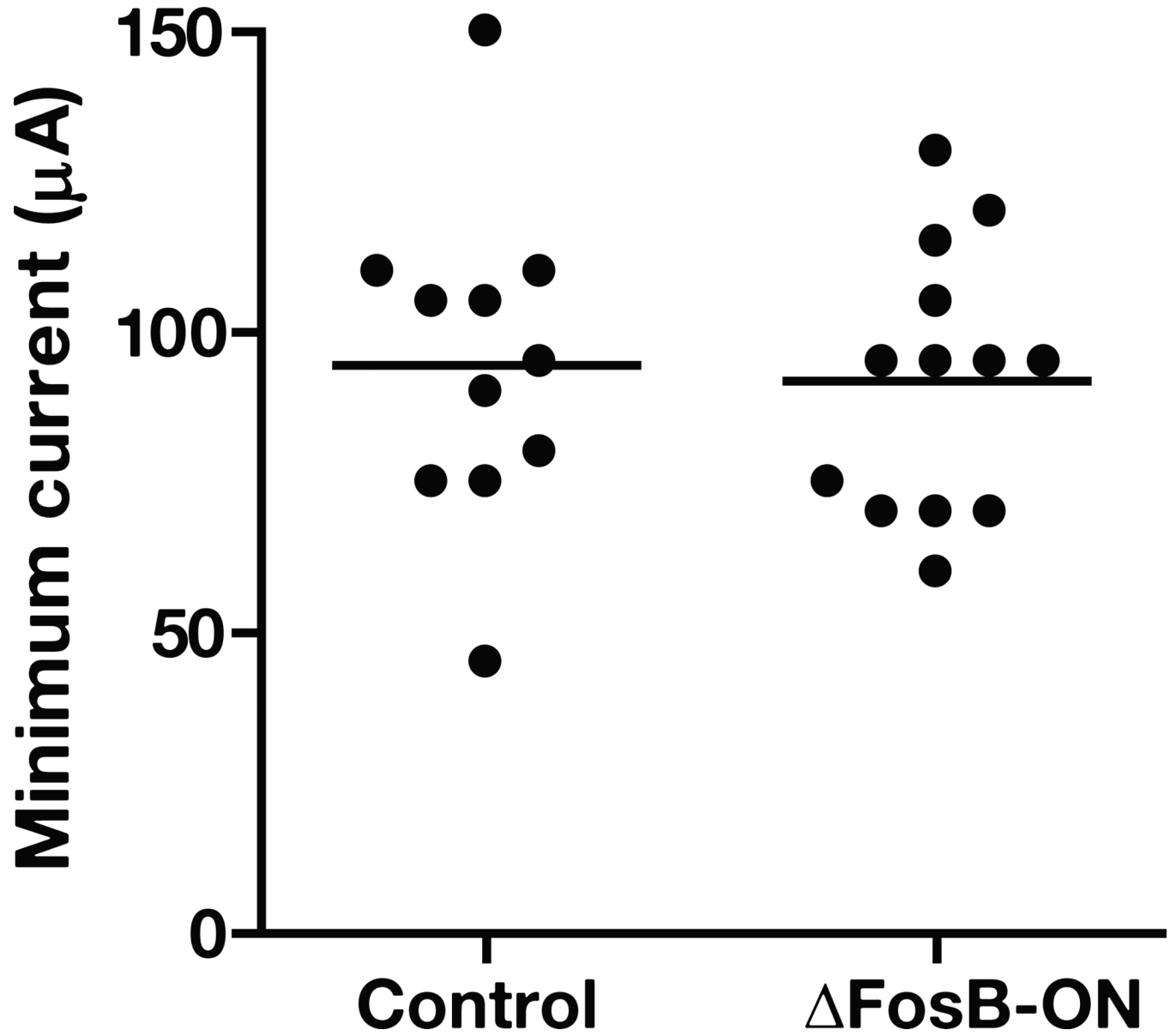

Figure 3.
Inducible ΔFosB overexpression has no effect on minimum current required to support ICSS. Scatterplot shows mean minimum current (bars) required to support robust ICSS behavior (60 ± 6 responses/min) in individual mice (filled circles) in the littermate control (on DOX, *n* = 11) or ΔFosB overexpression group (ΔFosB-ON, *n* = 13).

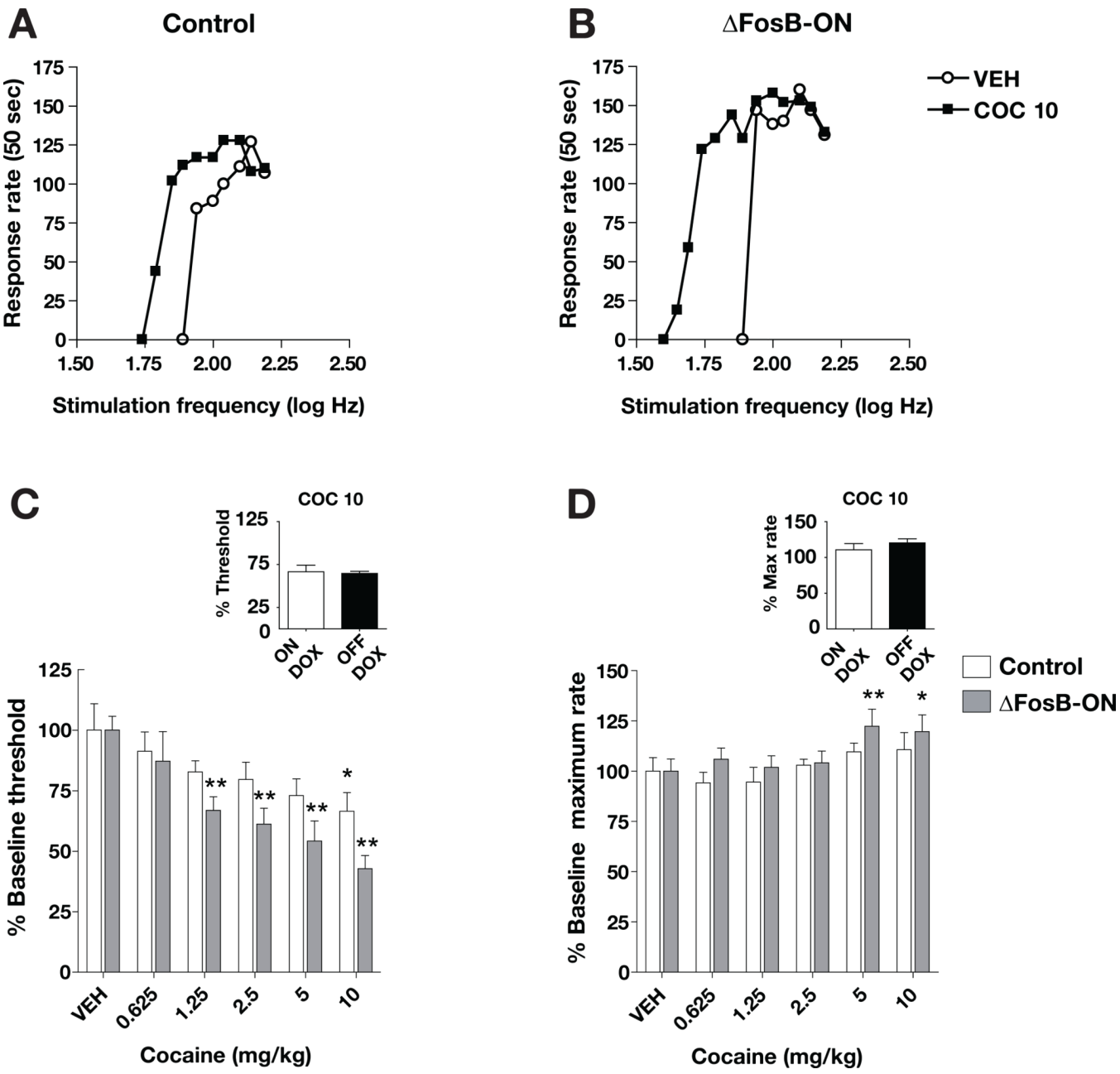

Figure 4.

Inducible ΔFosB overexpression enhances sensitivity to the rewarding effects of cocaine. **(A,B)** Rate-frequency functions for individual representative mice in each group demonstrate leftward shifts in both groups that are larger in ΔFosB-ON mice following the 10 mg/kg dose of cocaine (COC 10). **(C)** Cocaine reduces mean (±SEM) ICSS thresholds for cocaine in both Control (n = 7) and ΔFosB-ON (n = 8), but this effect was apparent in off-DOX mice at lower doses. Inset: Cocaine (10 mg/kg; COC 10) causes equivalent reductions in mean ICSS thresholds in ON-DOX or OFF-DOX Control mice. Means are expressed as a percent of baseline ICSS threshold. **(D)** ΔFosB-ON mice show increased mean maximum rates of responding apparent at the highest doses of cocaine used. *p<0.05, **p<0.01 for within-group comparisons to saline vehicle treatment (VEH). Inset: Cocaine (10 mg/kg; COC 10) causes no elevation of mean maximum rates of responding in ON-

DOX or OFF-DOX Control mice. Means are expressed as a percent of baseline maximum rate.

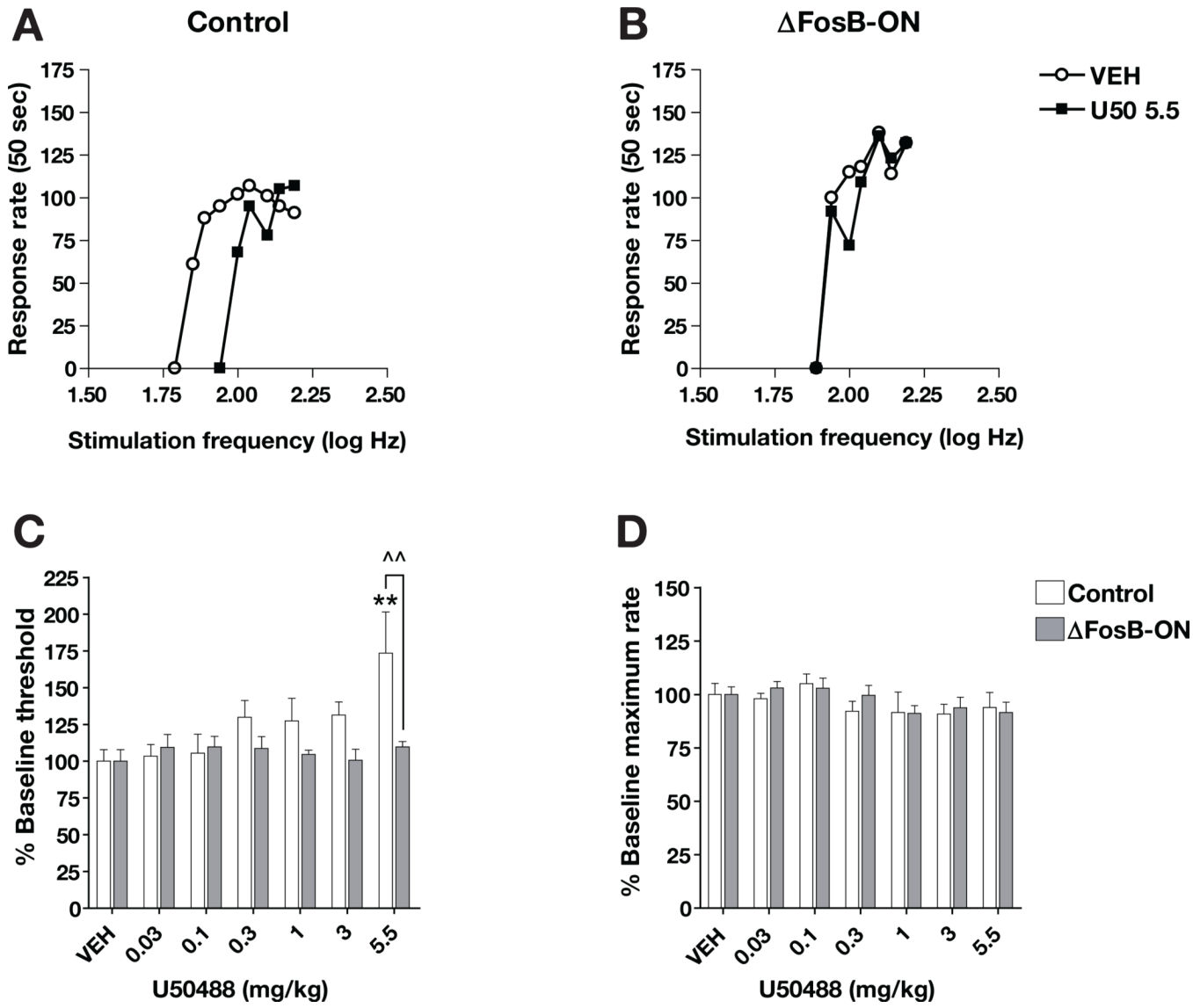

Figure 5.

Inducible ΔFosB overexpression blocks anhedonic effects of U50488. **(A,B)** Rate-frequency functions for individual representative mice in each group demonstrate rightward shifts only in Control mice following the 5.5 mg/kg dose of U50488 (U50 5.5). **(C)** U50488 increases mean (±SEM) ICSS thresholds for Control mice (n = 4), while ΔFosB-ON (n = 8) mice are unaffected. **(D)** Mean maximum rates of responding in both groups were unaffected. **p<0.01 for within-group comparisons, ^^p<0.01 for between-group comparisons.