

## REGULAR RESEARCH ARTICLE

# Reduction of Cocaine-Induced Locomotor Effects by Enriched Environment Is Associated with Cell-Specific Accumulation of $\Delta$ FosB in Striatal and Cortical Subregions

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## Abstract

**Background:** Early exposure to enriched environments has been shown to decrease the locomotor effects induced by repeated injections of cocaine and modify basal and cocaine-induced total protein levels of the transcription factor  $\Delta$ FosB in the whole striatum of mice. In this study, we aimed at characterizing whether the profile of  $\Delta$ FosB accumulation induced by enriched environments and cocaine would be similar or different in terms of brain areas and cell type.

**Methods:** We used mice expressing the eGFP protein in D1 receptor positive (D1R(+)) neurons to determine whether  $\Delta$ FosB induced by enriched environment or cocaine injections (5 × 15 mg/kg) would occur in selective subpopulations of neurons in several subregions of the striatum and prefrontal cortex.

**Results:** We found that: (1) exposure to enriched environment reduces cocaine-induced locomotor activation, confirming our previous findings; (2) exposure to enriched environment by itself increases the accumulation of  $\Delta$ FosB mostly in D1R(-) cells in the shell part of the nucleus accumbens and dorsal striatum, whereas in the nucleus accumbens core,  $\Delta$ FosB accumulates in both D1R(+) and D1R(-) neurons; (3) in standard environment mice, cocaine induces accumulation of  $\Delta$ FosB selectively in D1R(+) cells in the nucleus accumbens, dorsal striatum, and infralimbic cortex; and (4) the effects of enriched environments and cocaine on accumulation of  $\Delta$ FosB were reciprocally blocked by their combination.

**Conclusions:** Altogether, these results suggest that the enriched environment-induced reduction in behavioral effects of cocaine might result from 2 distinct effects on  $\Delta$ FosB in striatal medium-sized spiny neurons belonging to the direct and indirect pathways.

**Keywords:** enriched environment, cocaine,  $\Delta$ FosB, medium-sized spiny neurons, behavioral sensitization

## Significance Statement

Here, we show that the preventive effects of environmental enrichment on the development of behavioral sensitization are associated with cell-specific alterations in  $\Delta$ FosB levels in the striatum. Contrary to cocaine that increases  $\Delta$ FosB levels in neurons expressing dopamine D1 receptors, which constitute the direct pathway, environmental enrichment increases them in neurons lacking D1 receptors, which constitute the indirect pathway. Moreover, environmental enrichment inhibits cocaine-induced locomotor activation and increases in  $\Delta$ FosB levels. Therefore, by strengthening the indirect pathway, environmental enrichment opposes the addiction-related effects of cocaine. These results shed light on the neuroadaptations induced by environmental enrichment that participate in its beneficial effects. In addition, these results highlight the importance of cell-specific neuroadaptations that can lead to opposite functional effects.

## Introduction

Acute injection of cocaine increases locomotor activity in animals, and this effect sensitizes after repeated injections of the drug. This phenomenon is termed behavioral sensitization (Kalivas and Stewart, 1991), and it has been shown to be long-lasting (Vanderschuren and Kalivas, 2000) and to be associated with long-term neuroadaptations in the brain (Pierce and Kalivas, 1997). One of the molecular mechanisms involved in the behavioral sensitization is the accumulation of the transcription factor  $\Delta$ FosB in the striatum (Chen et al., 1997). Compared with other members of the Fos family protein, whose expression is rather transient, the expression of  $\Delta$ FosB is more stable (Chen et al., 1997) and, because of its stability,  $\Delta$ FosB accumulates in several brain regions, including the prefrontal cortex and striatum, thought to be involved in habit formation after chronic administration of cocaine (Hope et al., 1994; Nestler et al., 2001; McClung et al., 2004; Perrotti et al., 2008). For this reason,  $\Delta$ FosB is often considered as a molecular switch for addiction (Nestler et al., 2001).

In the striatum, most of the neurons are GABAergic medium-sized spiny neurons (MSNs) (95% in rodents) (Kawaguchi, 1997; Bolam et al., 2000; Tepper and Bolam, 2004; Bertran-Gonzalez et al., 2010). These neurons can be mainly classified in 2 subtypes of neurons according to the dopaminergic receptor subtype that they express. One subpopulation of MSNs expresses the D1 receptor (D1R) subtype and corresponds to neurons projecting directly to midbrain regions, thus constituting the direct pathway (Le Moine et al., 1991; Cerovic et al., 2013; Gangarossa et al., 2013). The second subpopulation of MSNs expresses the D2 receptor (D2R) subtype and corresponds to neurons projecting to the same midbrain regions through other structures such as the globus pallidus pars externa or the subthalamic nucleus, thus constituting the indirect pathway (Le Moine et al., 1990; Cerovic et al., 2013; Gangarossa et al., 2013). It is thought that these MSNs subtypes and their parallel pathways exert complementary, and sometimes opposite, actions on behaviors controlled by the cortico-striatal system (Gerfen and Surmeier, 2011). By use of optogenetic approaches, it was shown that activation of the direct pathway, corresponding to D1R(+) cells, increases locomotion and produces reinforcement, whereas activation of the indirect pathway, corresponding to D2R(+) cells, increases freezing behavior and does not produce reinforcement (Kravitz et al., 2010, 2012). In addition, the indirect pathway seems to play a role in aversive behavior (Hikida, et al., 2010), and strengthening of this pathway promotes resilience to cocaine compulsive use (Bock et al., 2013), whereas its inactivation increases the behavioral sensitization induced by amphetamine (Ferguson et al., 2011). Finally, activation of neurons of the direct pathway increases the rewarding effects of cocaine, whereas activation of neurons of the indirect pathway decreases them (Lobo et al., 2010). Importantly, the reactivity to cocaine of each subtype

of neurons of the striatum is also different. In fact, the D1R(+) MSNs are selectively activated in response to acute cocaine, as attested by the activation of the ERK pathway in these neurons (Bertran-Gonzalez et al., 2008), which is in agreement with the major involvement of the D1R subtype in the increased expression of immediate early genes induced by cocaine (Moratalla et al., 1996; Lee et al., 2006). After chronic cocaine, accumulation of  $\Delta$ FosB is mostly observed in MSNs of the direct pathway in the nucleus accumbens (NAc) and dorsal striatum (DSt) (Lobo et al., 2013). Interestingly, the inducible overexpression of  $\Delta$ FosB selectively in D1R(+) MSNs of the striatum in transgenic mice seems to reproduce cocaine's long-term effects such as increased sensitivity to the locomotor-activating and rewarding effects of drugs (Kelz et al., 1999; McClung et al., 2004); these mice also show an increased motivation for cocaine in a progressive ratio schedule of self-administration (Colby et al., 2003). These results suggest a major involvement of neurons of the direct pathway in behavioral and neuronal adaptations induced by drugs.

Environmental enrichment (EE) has been shown to reduce the behavioral and neurobiological effects of drugs such as cocaine (Solinas et al., 2009) and heroin (El Rawas et al., 2009), but not methamphetamine (Thiriet et al., 2011). In particular, exposing mice to enriched conditions during adolescence reduces the activating effects of cocaine in a behavioral sensitization protocol compared with controls animals housed in standard environments (SE) (Solinas et al., 2009). In parallel to these behavioral effects, EE was found to produce drastic changes in the accumulation of  $\Delta$ FosB induced by cocaine in the striatum. In fact, EE by itself increased levels of  $\Delta$ FosB in striatum (Solinas et al., 2009; Venebra-Munoz et al., 2014; Zhang et al., 2014) and prefrontal cortex (Lehmann and Herkenham, 2011; Venebra-Munoz et al., 2014). In addition,  $\Delta$ FosB levels were comparable in EE mice injected repeatedly with cocaine and control SE animals injected with saline, suggesting that, in contrast to what is observed in SE mice, cocaine promotes degradation of  $\Delta$ FosB protein in EE mice (Solinas et al., 2009; Zhang et al., 2014). Although previous studies suggest a role for  $\Delta$ FosB in the decreased behavioral effects of cocaine found in EE mice, it is not clear how similar increases in striatal  $\Delta$ FosB levels could lead to opposite effects. A recent study by Lobo and colleagues (2013) has started to provide insights into these apparent paradoxical effects. In that study, it was shown that exposure to EE, in contrast to what is observed in response to cocaine, increased the expression of  $\Delta$ FosB in both D1R(+) and D2R(+) cells in NAc (shell and core) and DSt, suggesting that the neurons targeted by cocaine and by EE are not the same. However, they did not compare the accumulation of  $\Delta$ FosB in response to cocaine as a function of differential environmental conditions.

In this study, we investigated whether the pattern of  $\Delta$ FosB accumulation in response to chronic cocaine differs in EE

compared with SE mice. We used transgenic mice that express the enhanced green fluorescent protein (eGFP) specifically in DR1(+) cells, which allowed for dissociation of the direct dopamine pathway from the indirect pathway. Mice were housed in EE or SE from weaning to adulthood and then underwent a behavioral sensitization protocol (5 injections of 15 mg/kg cocaine every second day). On the day after the last injection, brains were obtained for immunohistochemistry staining. We investigated  $\Delta$ FosB accumulation in different subregions of the striatum and prefrontal cortex, as these regions play role in behavioral sensitization and addiction (Vanderschuren and Kalivas, 2000).

## Materials and Methods

### Subjects

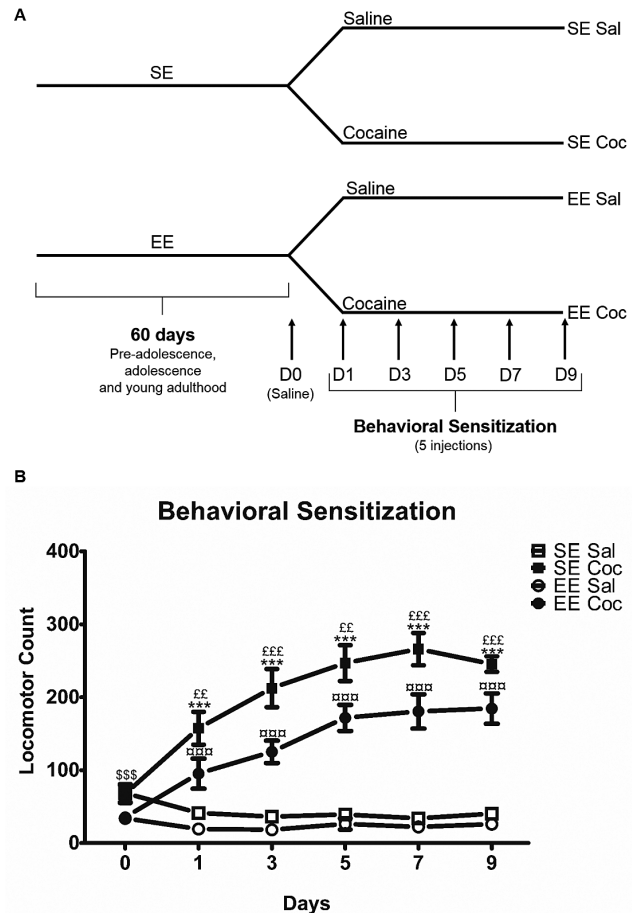
BAC heterozygous *Drd1a-eGFP* mice generated by GENSAT (Gene Expression Nervous System Atlas) at Rockefeller University (New York, NY) backcrossed with C57BL/6 line (Gong et al., 2007) were used in this study (male genitors were kindly provided by Drs D. Hervé and J. A. Girault). In these mice, the expression of the eGFP protein is driven by the D1R gene regulatory elements to identify cells expressing the D1R subtype, which are labeled in green. Mice were housed in a temperature-controlled environment on a 12-h-light/-dark cycle with the lights on from 7:00 AM to 7:00 PM and had ad libitum access to food and water. All experiments were conducted during the light phase, were in accordance to European Union directives (2010/63/EU) for the care of laboratory animals, and were approved by the local ethical committee (COMETHEA no. 02469-01).

### Housing Environmental Conditions

After weaning (3 weeks of age), mice were randomly divided into 2 different housing environmental conditions: SE or EE. SE cages were common housing cages (25 × 20 × 15 cm) and EE cages consisted of larger (60 × 38 × 20 cm) cages containing a running wheel and a small plastic house, and 4 toys that were changed once a week with new toys of different shapes and colors. For both SE and EE conditions, mice were housed in groups of 4 for 2 to 3 months before the start of the behavioral experiments.

### Locomotor Activity and Behavioral Sensitization Procedure

Horizontal locomotion was measured by the number of beam crossings in motor chambers (19 × 11 × 14 cm) (www.imetronic.com) connected to a computer (Solinas et al., 2009). A schematic representation of the protocol used for the behavioral sensitization is presented in Figure 1A. On the first day (day 0), all mice were injected with saline and placed in the locomotor chamber for 60 minutes to evaluate their basal locomotor activity. The next day, mice were placed again for 30 minutes in the locomotor chamber for habituation. After this period, one-half of the mice were injected with cocaine (15 mg/kg, i.p.) and the other one-half with saline (NaCl 0.9 g/L, i.p.), and they were immediately placed back in the same locomotor chamber for a 60-minute period during which their locomotor activity was measured. Then, every second day (5 injections in total), mice were submitted to the same protocol (Figure 1A). Four groups of animals were obtained (n = 15–16/group): SE Sal, SE Coc, EE Sal, and EE Coc.



**Figure 1.** Behavioral sensitization to cocaine in D1R-eGFP mice reared in standard (SE) or enriched (EE) environments. (A) Schematic representation of the experimental design used for the behavioral sensitization protocol. (B) Development of behavioral sensitization to cocaine (15 mg/kg, i.p.). Mice exposed to SE and EE develop behavioral sensitization, that is, cocaine-induced locomotor activity increases over days, but EE consistently show reduced locomotor response to cocaine compared with SE. We used Fisher's protected least-squares difference posthoc test: \$\$\$  $P < .001$  indicates different responses to saline of SE and EE mice on day 0; \*\*\*  $P < .001$  indicates difference between SE Sal and SE Coc groups; □□□  $P < .001$  indicates difference between EE Sal and EE Coc groups and EE  $P < .01$  and EEE  $P < .001$  indicates difference between SE Coc and EE Coc groups. Results represent means  $\pm$  SEM from 15 to 16 mice.

### Immunohistochemistry

#### Tissue Preparation

Eighteen to 20 hours after the last injection of cocaine or saline, mice were rapidly and deeply anaesthetized with pentobarbital (500 mg/kg, i.p., Sanofi-Aventis). We chose this time interval of brain collection to ensure that we are only detecting  $\Delta$ FosB protein after chronic cocaine administrations and not other forms of FosB (Hope et al., 1994; McClung et al., 2004; Perrotti et al., 2008). Mice were then transcardially perfused with 4% paraformaldehyde dissolved in 0.1 M sodium phosphate buffer (pH 7.4). Brains were then removed and postfixed in 4% paraformaldehyde for 4 hours and stored in 30% sucrose at 4°C until sectioning. All serial brain sections (40  $\mu$ m) were then cut using a freezing microtome (Leica RM2145, www.leica-microsystems.com) and stored in cryo-protective solution (glycerol 20%, DMSO 2%, NaCl 0.9%, PB 0.1 M) at -20°C until processed for immunolabeling.

### Immunolabeling

Free-floating sections obtained from 8 to 10 mice of each group (SE Sal, SE Coc, EE Sal, and EE Coc) were processed for  $\Delta$ FosB and eGFP protein detection. Sections taken from the prefrontal cortices to the striatum (+1.98 to +1.1 mm from bregma) were submitted to immunolabeling. Sections were initially washed extensively in phosphate buffered saline (PBS) ( $3 \times 10$  minutes) and incubated for 2 hours in blocking solution (PBS containing 3% bovine serum albumin [Sigma-Aldrich] and 0.3% Triton X-100). Subsequently, sections were incubated for 24 hours at room temperature with 2 primary antibodies diluted in the blocking solution: a rabbit monoclonal anti-FosB antibody (1:2000, 14695S, Cell Signaling) and a goat polyclonal anti-eGFP antibody (1:2000, Ab6673, Abcam) to amplify the eGFP signal. Although the anti-FosB antibody recognizes both FosB and  $\Delta$ FosB proteins, previous studies showed that the expression of other Fos and Fra proteins disappears by 18 hours after the last cocaine administration, because they are rapidly degraded, unlike FosB, which is more stable and accumulates in brain areas after cocaine chronic administration (Hope et al., 1994; McClung et al., 2004; Perrotti et al., 2008). This is in agreement with our previous results using western-blot approaches (Solinas et al., 2009). Sections were then washed in PBS ( $3 \times 10$  minutes) and incubated for 45 minutes at room temperature with secondary antibodies diluted in the blocking solution: donkey anti-rabbit coupled to AlexaFluor555 (1:500, A31572, Life Technologies) and a donkey anti-goat coupled to AlexaFluor488 (1:500, A11055, Life Technologies). Sections were rinsed in PBS ( $2 \times 10$  minutes) and with a phosphate buffer (0.1 M, 10 minutes). Finally, sections were mounted onto gelatin-coated slides, dried, and dehydrated before cover slipping. Negative control sections were incubated in blocking solution without primary antibody (not shown).

### Analysis of the Density of $\Delta$ FosB(+) Cells

The colocalization of  $\Delta$ FosB labeling with the eGFP labeling (i.e., D1R(+) neurons) in the same cell was evaluated using confocal microscopy (confocal laser-scanning microscope FV1000, Olympus). For quantification of  $\Delta$ FosB(+) cells, images were acquired with the Axio Imager M2 microscope with Apotome.2 (Carl Zeiss) ( $\times 20$ ). The number of cells expressing  $\Delta$ FosB was quantified using Image J software in 7 regions of the brain bilaterally. Two to 3 sections were analyzed for each animal and for each region, including the prelimbic cortex (PrL) and the infralimbic cortex (IL) (corresponding to sections from +1.98 to +1.70 mm from bregma), the anterior cingulate cortex (ACC), the NAc (shell and core), and the DSt, medial and lateral parts (corresponding to sections from +1.42 to +1.10 mm from bregma). We initially counted the total number of  $\Delta$ FosB(+) cells in each brain area (see supplementary Figure 2 for delineation) to evaluate any changes in their density expressed as cells/mm<sup>2</sup>. To this extent, we fixed an arbitrary threshold using slides from a saline animal; this threshold corresponds to the half value of the maximal red signal. Cells with mean intensity above this threshold were considered  $\Delta$ FosB(+) in sections from all other animals blindly counted. We then counted the number of  $\Delta$ FosB(+) cells in which labeling corresponding to the eGFP protein was also detected in order to determine whether  $\Delta$ FosB accumulates selectively in D1R(+) cells, in D1R(-) cells, or in both. In the striatum, MSNs correspond to about 95% of all neurons; consequently, we could consider that the cells without eGFP labeling in which  $\Delta$ FosB accumulates are probably MSNs expressing the D2R subtype; however, to be more accurate, we described them as D1R(-) cells.

### Statistical Analysis

All results are presented as group means ( $\pm$ SEM). Differences in behavioral activity and in the number of cells expressing  $\Delta$ FosB between groups were assessed by 1-, 2-, or 3-way ANOVA. Results showing significant overall changes were subjected to Fisher protected least-squares difference posthoc test. Differences were considered significant when  $P < .05$ .

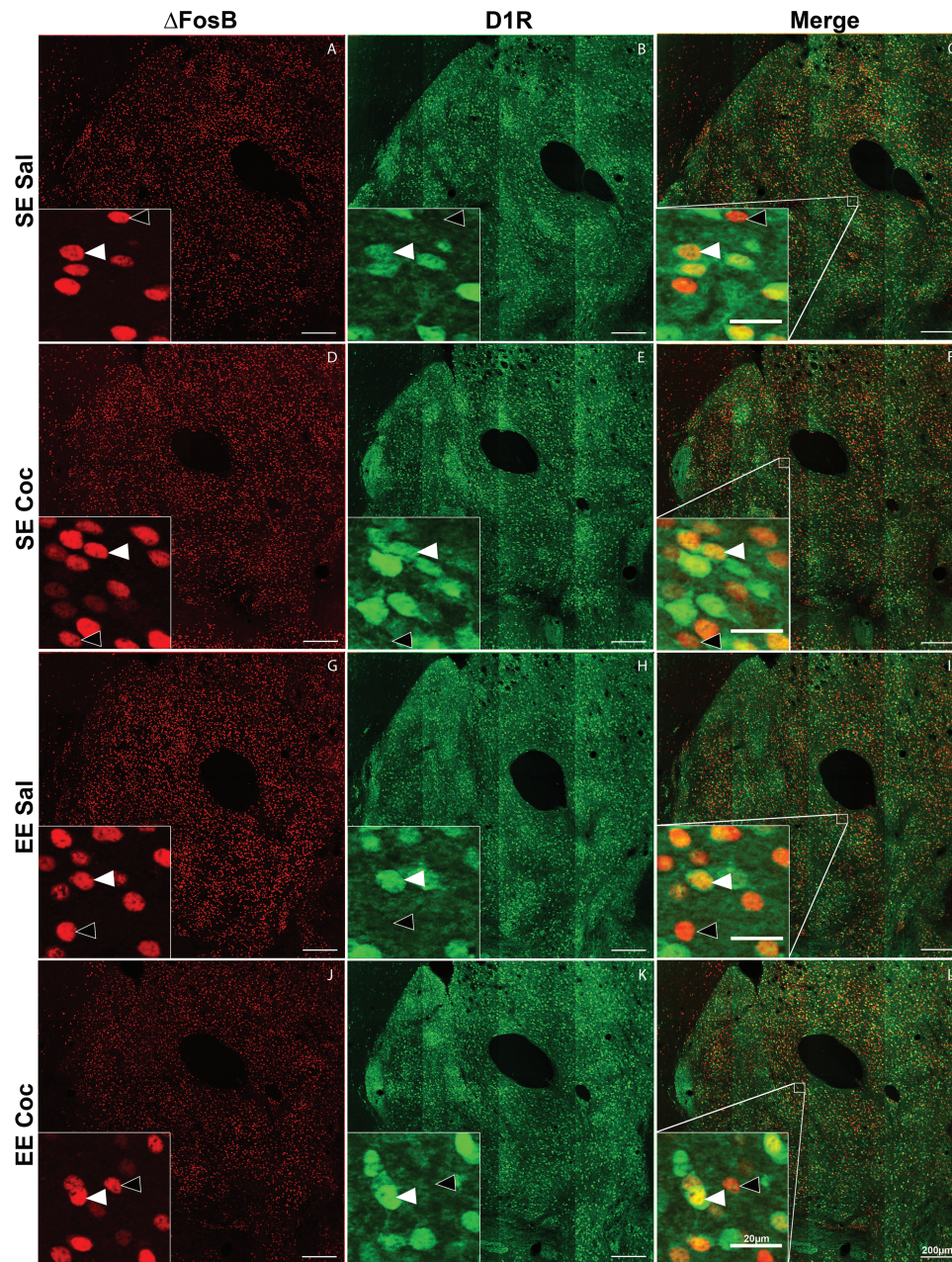
## Results

### EE Reduces the Sensitivity to the Locomotor Effects Induced by Repeated Injections of Cocaine in *Drd1a*-eGFP Mice

Prior to the first cocaine injection, basal levels of locomotor activity were higher in SE mice compared with EE mice ( $P < .001$ ). Further, as previously reported in C57BL/6 (Bezard et al., 2003), the first injection of cocaine (15 mg/kg, i.p.) produced significantly higher locomotor activation (40%,  $P < .01$ ) in the SE group compared with the EE group in BAC *Drd1a*-eGFP mice (Figure 1B). With repeated administrations, both SE Coc and EE Coc groups developed significant sensitization; for both groups the effect of cocaine increased upon repeated administration (Figure 1B). The relative amplitude of the sensitization as measured by the mean of the activities at days 7 and 9 (corresponding to the maximal response to cocaine) over the activity at day 1 was similar in both SE and EE mice (supplementary Figure 1). However, the locomotor effects of cocaine were consistently lower (about 40%) in EE compared with SE mice (Figure 1B) (3-way ANOVA, EE,  $F(1,57) = 16.02$ ,  $P < .01$ ; treatment effect,  $F(1,57) = 197.83$ ,  $P < .0001$ ; day effect,  $F(4, 228) = 15.83$ ,  $P < .0001$ ; environment  $\times$  treatment interaction,  $F(1,57) = 6.73$ ,  $P < .05$ ; treatment  $\times$  day interaction,  $F(4,228) = 14.85$ ,  $P < .0001$ ). These results provide additional evidence, using transgenic mice, that EE exposure can reduce the activating effects of cocaine in a behavioral sensitization protocol.

### $\Delta$ FosB Accumulation in Subregions of the Striatum after Chronic Cocaine in SE and EE Mice

Figure 2 illustrates the labeling obtained in NAc core with antibodies against the eGFP protein (green labeling) (Figure 2, middle and right column), which allowed identifying the cells expressing D1R and against  $\Delta$ FosB protein (red labeling) (Figure 2, left and right column) in mice of the 4 groups: SE Sal (Figure 2A-C), SE Coc (Figure 2D-F), EE Sal (Figure 2G-I), and EE Coc (Figure 2J-L). Consistent with a previous report (Lobo et al., 2013), exposure to EE or injections of cocaine did not alter the number of D1R(+) cells (data not shown). The number of  $\Delta$ FosB(+) cells and  $\Delta$ FosB/D1R(+) cells was quantified in 4 regions of the striatum: medial DSt, lateral DSt, NAc shell, and NAc core (Figure 3A, left to right). The delimitations of the surface in which cells were counted for each brain area are presented in Figure 3A and supplementary Figure 2. In saline controls, exposure to EE increased the density of total  $\Delta$ FosB(+) cells (+21%,  $P < .05$  in medial DSt; +26%,  $P < .05$  in NAc shell; and +31%,  $P < .001$  in NAc core), but not in lateral DSt compared with SE saline controls (Figure 3B). When we looked at specific cell type, this effect was mainly observed for D1R(-) cells (+35%,  $P < .05$  in lateral DSt; +40%,  $P < .05$  in medial DSt; +38%,  $P < .01$  in NAc shell), except for NAc core, in which the increase was observed both in D1R(+) cells (+25%,  $P < .01$ ) and D1R(-) cells (+43%,  $P < .01$ ). In SE mice, we found a significant cocaine-induced increase in the density of total  $\Delta$ FosB(+) cells in all these



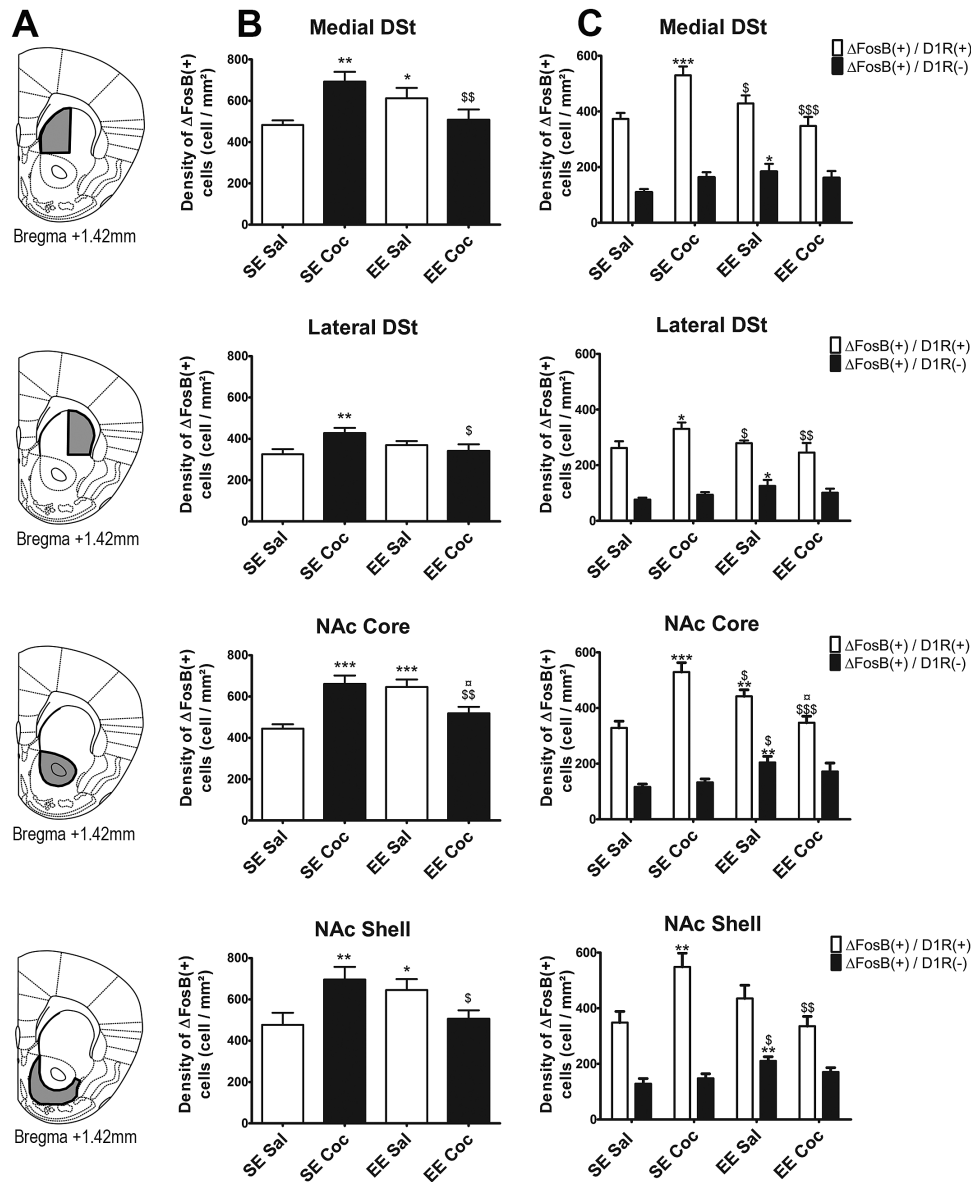
**Figure 2.** Representative examples of immunostaining obtained at the level of NAc core in standard environment (SE) Sal, SE Coc, enriched environment (EE) Sal, and EE Coc groups of *D1R-eGFP* mice. (A, D, G, and J) Immunolabeling against  $\Delta$ FosB in red; (B, E, H, and K) immunolabeling against enhanced green fluorescent protein (eGFP) in green; (C, F, I, and L) the merge of the 2 labeled in orange. Orange cells correspond to cells that expressed both  $\Delta$ FosB and eGFP. Images were taken at the level of Bregma +1.42 mm. Scale bars = 200  $\mu$ m. The insets in each image show enlargement of regions indicated by open squares on images C, F, I, and L. White filled arrowheads point to  $\Delta$ FosB(+)/*D1R*(+) cells and unfilled arrowheads point to  $\Delta$ FosB(+)/*D1R*(-) cells. Scale bar in insets = 20  $\mu$ m.

striatal regions (Figure 3B) (+30%,  $P < .001$  in medial DST; +24%,  $P < .001$  in lateral DST; +31%,  $P < .001$  in NAc shell; and +32%,  $P < .01$  in NAc core). In contrast to the effects of EE, however, repeated administration of cocaine in SE mice increased  $\Delta$ FosB expression selectively in *D1R*(+) neurons (Figure 3C) (+29%,  $P < .001$  in medial DST; +24%,  $P < .05$  in lateral DST; +36%,  $P < .01$  in NAc shell; and +37%,  $P < .001$  in NAc core). Finally, in EE mice, cocaine did not increase the density of  $\Delta$ FosB(+) cells in any of the striatal regions investigated, and it also reversed the accumulation of  $\Delta$ FosB observed in EE control mice (Figure 3B). It should be noted that in all groups of mice, the density of  $\Delta$ FosB/*D1R*(+)

cells was always higher than the density of  $\Delta$ FosB/*D1R*(-) cells (mean difference for all groups: +175%,  $P < .0001$  in medial DST; +178%,  $P < .0001$  in lateral DST; +160%,  $P < .0001$  in NAc shell; and +166%,  $P < .0001$  in NAc core).

#### $\Delta$ FosB Accumulation in Subregions of the Prefrontal Cortex after Chronic Cocaine in SE and EE Mice

Figure 4A presents the different cortical subareas (PrL, IL, and ACC) in which expression of  $\Delta$ FosB(+) cells was quantified. The delimitations of the surface in which cells were counted



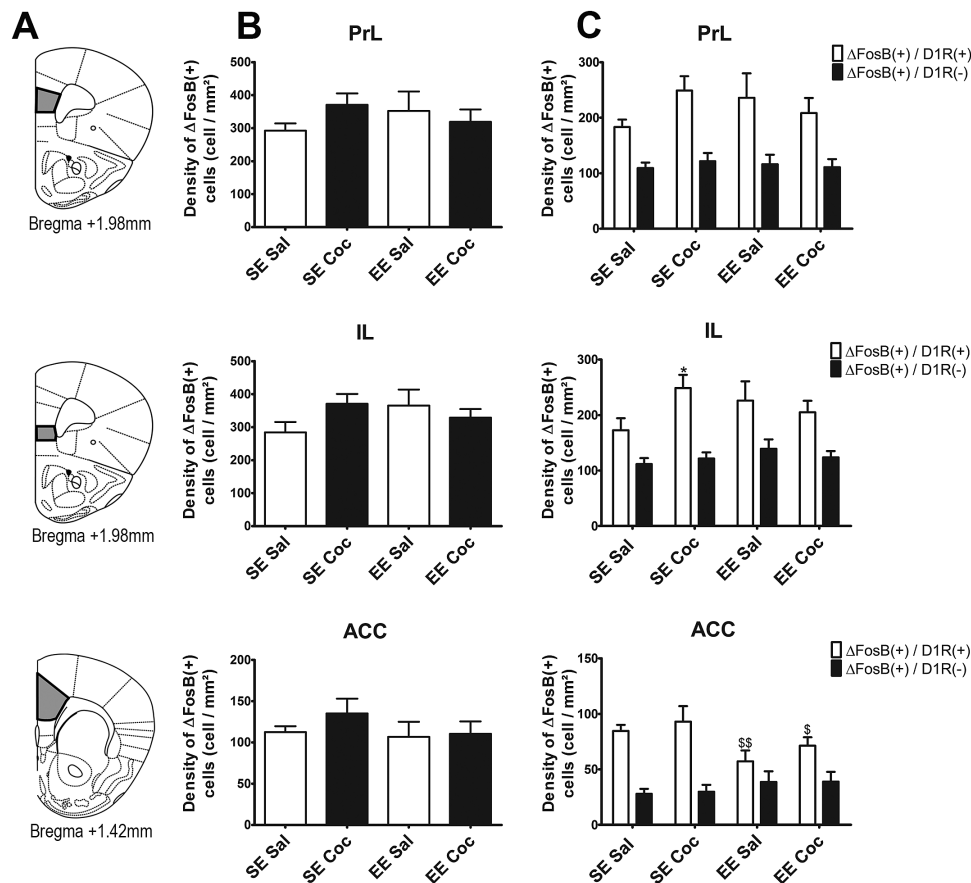
**Figure 3.** Density of  $\Delta$ FosB(+) cells in the striatum of standard environment (SE) Sal, SE Coc, enriched environment (EE) Sal, and EE Coc mice and characterization of the phenotype of these cells (D1 receptor [D1R](+) or D1R(-)). (A) Delimitations of the 4 striatal subregions (medial dorsal striatum [DSt], lateral DSt, nucleus accumbens [NAc] core and shell) used for quantification. (B) Density of  $\Delta$ FosB(+) cells in each subregion. (C) Density of  $\Delta$ FosB(+)/D1R(+) cells (white bars) and  $\Delta$ FosB(+)/D1R(-) cells (black bars) in each subregion. Cocaine treatment significantly increased the density of  $\Delta$ FosB(+)/D1R(+) MSNs in the 4 regions. EE significantly increased the density of  $\Delta$ FosB(+)/D1R(-) cells in the medial and lateral DSt and NAc shell. In the NAc core, EE significantly increased the density of both  $\Delta$ FosB/D1R(+) and  $\Delta$ FosB(+)/D1R(-) cells. The density of  $\Delta$ FosB/D1R(+) and  $\Delta$ FosB(+)/D1R(-) cells were similar in EE Coc and SE Sal animals. Results are expressed as means  $\pm$  SEM ( $n=8-10$ /group). We used Fisher's protected least-squares difference posthoc test: \*  $P < .05$ , \*\*  $P < .01$ , and \*\*\*  $P < .001$ , difference compared with SE Sal; \$  $P < .05$ , \$\$  $P < .01$ , and \$\$\$  $P < .001$ , difference compared with SE Coc; □  $P < .05$ , \$\$\$  $P < .001$ , difference compared with EE Sal.

for each brain area are presented in [Figure 4A](#). We found no effect of the rearing environment or of repeated administration of chronic cocaine on the global density of  $\Delta$ FosB(+) cells across groups ([Figure 4B](#)). However, in the IL, we found an increase in the density of  $\Delta$ FosB/D1R(+) in SE mice exposed to repeated cocaine (+30%,  $P < .05$ ) and this effect was blunted by EE ([Figure 4C](#)). Interestingly, in the ACC, EE decreased the density of  $\Delta$ FosB/D1R(+) cells (-37%,  $P < .01$ ), but did not change the density of  $\Delta$ FosB/D1R(-) cells (a significant DA subtype  $\times$  environment interaction ( $F(1,58) = 10.27$ ,  $P < .01$ ) ([Figure 4C](#)). As found in the striatal subregions, the density of  $\Delta$ FosB/D1R(+) cells was always higher than the density of  $\Delta$ FosB/D1R(-) cells

in all groups (mean difference for all groups: +128%,  $P < .0001$  in PrL; +71%,  $P < .0001$  in IL; +128%,  $P < .0001$  in ACC).

## Discussion

The striatum is a relatively heterogeneous brain region that anatomically and functionally appears to have a gradient from the more ventral and medial part (NAc shell), passing from intermediate zones (NAc core and medial DSt) to the more dorsal and lateral part (lateral DSt) that are respectively involved in limbic, associative, and sensorimotor functions ([Le Moal and Simon, 1991](#); [Voorn et al., 2004](#); [Ikemoto et al., 2015](#)). In this study, we



**Figure 4.** Density of total  $\Delta$ FosB(+) cells in the prefrontal cortex of standard environment (SE) Sal, SE Coc, enriched environment (EE) Sal, and EE Coc mice and characterization of the phenotype of these cells (D1 receptor [D1R](+) or D1R(-)). (A) Illustrations of the 3 cortical subregions (prelimbic [PrL], infralimbic [IL], and the anterior cingulate [ACC]) used for quantification. (B) Density of  $\Delta$ FosB(+) cells in each subregion. (C) Density of  $\Delta$ FosB(+)/D1R(+) cells (white bars) and  $\Delta$ FosB(+)/D1R(-) cells (black bars) in each subregion. Cocaine treatment did not significantly modulate the overall density of  $\Delta$ FosB(+) cells in the 3 regions; however, it increased the density of  $\Delta$ FosB(+)/D1R(+) cells in the IL of SE mice, an effect blunted in EE mice. EE significantly decreased the density  $\Delta$ FosB(+)/D1R(+) cells in the ACC, independently of cocaine treatment. Results are expressed as means  $\pm$  SEM ( $n=8-10$ /group). We used Fisher's protected least-squares difference posthoc test: \*  $P < .05$ , difference compared with SE Sal; \$  $P < .05$  and \$\$  $P < .01$ , difference compared with SE Coc.

found that the profile of the effects of cocaine and EE were relatively similar in the 4 regions of the striatum analyzed, but the magnitude of these effects was larger in the ventral compared with dorsal areas (Figure 3B). This is consistent with the general finding that the ventral striatum is more sensitive to external events such as drug self-administration (Perrotti et al., 2008) and environmental manipulations (Lobo et al., 2013).

In the present study, we found that chronic cocaine increases the levels of  $\Delta$ FosB in all striatal regions of SE mice, an effect that is likely due to the repeated overstimulation of dopaminergic neurons secondary to cocaine-induced increases of extracellular dopamine (Chen et al., 1997; Pontieri et al., 1995). Consistent with previous findings (Lobo et al., 2013), cocaine-induced accumulation of  $\Delta$ FosB was selective for neurons expressing the D1 subtype of dopamine receptors in all striatal regions. These findings are also consistent with previous studies demonstrating that acute cocaine induces phosphorylation of several intracellular signaling proteins restricted to D1R(+) MSNs (Bateup et al., 2008; Bertran-Gonzalez et al., 2008) and that the expression of Fos-related protein in response to cocaine depends on the D1R subtype (Moratalla et al., 1996; Lee et al., 2006). This is also in agreement with previous work showing that  $\Delta$ FosB accumulation in D1R(+) MSNs plays a major role in cocaine reward (Kelz et al., 1999; Colby et al., 2003; Grueter et al., 2013) and that the activation of D1R(+) cells in the

striatum produces reinforcing effects (Kravitz et al., 2012; Ikemoto et al., 2015). Therefore, the accumulation of  $\Delta$ FosB in the D1R(+) cells in the striatum may represent a long-lasting adaptation that strengthens the direct pathway and increases the addiction-inducing effects of repeated injections of cocaine.

Consistent with previous studies (Solinas et al., 2009; Lobo et al., 2013), EE by itself increased  $\Delta$ FosB levels in the striatum. Interestingly, in contrast to what was found with cocaine, especially in the NAc Shell,  $\Delta$ FosB levels were selectively increased in D1R(-) cells, which are likely D2R(+) MSNs. This finding contrasts with the study by Lobo et al. (2013), who reported that enrichment resulted in nonselective increased  $\Delta$ FosB levels in both D1R(+) and D2R(+) MSNs (Lobo et al., 2013). The reason for this discrepancy is not clear, but it could be due to differences in EE conditions such as the length of exposure to EE (4 weeks for Lobo et al. and 8 weeks in our study), the age of the animals (around postnatal day 50 for Lobo et al. and around postnatal day 78 in our study), and differences in the behavioral manipulation (injections in the home-cage for Lobo et al. and in the locomotor activity apparatus in our study). These experimental differences can also explain the fact that whereas in the paper by Lobo et al. D1R(+) and D2R(+) show similar levels of expression of  $\Delta$ FosB under basal condition, we found significantly more  $\Delta$ FosB/D1R(+) than  $\Delta$ FosB/D1R(-) cells. Regardless of these differences,

the general finding in both Lobo et al. (2013) and the current report is that EE produced qualitatively different effects on striatal  $\Delta$ FosB expression compared with the selective cocaine-induced increase  $\Delta$ FosB levels in D1R MSNs. Stimulation of D2R(+) MSNs neurons appears to have effects that are opposite to stimulation of D1R(+) MSNs neurons; for example, in contrast to D1R stimulation, D2R stimulation produces aversive effects (Kravitz et al., 2012; Ikemoto et al., 2015). Therefore, elevated levels of  $\Delta$ FosB in D2R(+) MSNs may strengthen this pathway and oppose cocaine's reinforcing effects. Indeed, it has been shown that activation of D2R(+) MSNs reduces the reinforcing effects of cocaine (Bock et al., 2013). These observations may explain the decreased locomotor reactivity to cocaine observed in EE mice (present study and Bezard et al., 2003) as well as the reduced locomotor effects induced by repeated cocaine administrations we found in EE mice (present study and Solinas et al., 2009).

Consistent with previous findings in the whole striatum (Solinas et al., 2009; Venebra-Munoz et al., 2014; Zhang et al., 2014), we found that: (1) exposure to EE prevented cocaine-induced increases in  $\Delta$ FosB levels in the NAc shell and core and in the medial DSt and (2) striatal  $\Delta$ FosB levels were reduced in cocaine-treated EE mice compared with saline-treated EE mice. Thus, the inability of cocaine to increase  $\Delta$ FosB levels in the striatum of EE mice may explain the reduced behavioral effects (present study and Solinas et al., 2009) and reward (Solinas et al., 2009) produced by cocaine in these mice. Conversely, cocaine reversed the accumulation of  $\Delta$ FosB induced by EE in D1R(-) MSNs neurons. Thus, EE and cocaine appear to antagonize each other's effects. Similarly, Robinson and Kolb (2004) found that both cocaine and EE increased dendritic arborization in the striatum but that previous exposure to cocaine prevented the ability of EE to produce this effect (Robinson and Kolb, 2004).

We also investigated the effects of cocaine and EE on  $\Delta$ FosB levels in 3 different subregions of the medial prefrontal cortex: the PrL, the IL, and the ACC, which are all involved in behavioral sensitization to cocaine (Schmidt et al., 1999; Tzschentke and Schmidt, 1999). In our study, cocaine produced a significant increase in  $\Delta$ FosB levels in D1R(+) but not in D1R(-) neurons in the IL, and EE did not increase  $\Delta$ FosB levels but decreased them in the ACC regardless of cocaine treatment. Previous studies (Perrotti et al., 2008; Winstanley et al., 2009) found increases in  $\Delta$ FosB levels in different subregions of the prefrontal cortex in response to cocaine (although their brain region distinctions were somewhat less precise than ours), but these increases were less pronounced than in the striatum. The fact that we were unable to detect significant cocaine-induced increases in  $\Delta$ FosB levels in cortical regions may be related to the fact that our protocol of cocaine exposure was less intense than those used in these studies (Perrotti et al., 2008; Winstanley et al., 2009). D1R(+) neurons have been shown to be expressed in cortical layers II, V, and VI (Vincent et al., 1993, 1995; Gaspar et al., 1995) and in both nonpyramidal (Vincent et al., 1993, 1995) and pyramidal neurons (Seong and Carter, 2012) that project to several brain regions, including the cortex itself, striatum, thalamus (Gaspar et al., 1995), and amygdala (Land et al., 2014). Future studies will be needed to identify the cortical cell type in which  $\Delta$ FosB levels were affected by cocaine and EE and to characterize their functional consequences on behavior.

Compared with the striatum, little is known about the consequences of increased  $\Delta$ FosB in IL on cocaine addiction, and therefore, the interpretation of these findings is not straightforward. In fact, only one study investigated the effects of EE on basal  $\Delta$ FosB levels in the cortex and found an increase in IL, PrL, and ACC (Lehmann and Herkenham, 2011). In contrast, in our study, EE did not affect  $\Delta$ FosB levels in the IL and PrL, and it even

decreased  $\Delta$ FosB levels in the ACC. These differences may be due to differences in the protocol of EE exposure, such as the duration and the age of mice, as well as the fact that Lehman and Herkenham (2011) housed control animals singly, whereas they were housed in groups of 4 in the current study. However,  $\Delta$ FosB increases have been reported after exposure to stressors such as social defeat (Hinwood et al., 2011; Lehmann and Herkenham, 2011; Vialou et al., 2014) and after opiate sensitization (Kaplan et al., 2011), which could suggest that this neuroadaptation participates in a negative emotional state that can render individuals vulnerable to drug addiction. Therefore, it is of interest that exposure to EE prevents this accumulation of  $\Delta$ FosB and probably the establishment of such a negative emotional state. Future studies are needed to determine whether increases of  $\Delta$ FosB levels in IL participate in the development and maintenance of addiction-related behaviors.

In conclusion, our study shows that both cocaine and EE increases  $\Delta$ FosB levels in the striatum, especially in the NAc. However, their profile of  $\Delta$ FosB induction is very different, and indeed opposite. Our results suggest that EE-induced increases in the levels of  $\Delta$ FosB in putative D2R(+) neurons of the striatum may result in potentiation of the indirect dopamine pathway and ultimately in the blunting of effects of cocaine that work through the direct dopamine pathway. Furthermore, we found that EE increases in  $\Delta$ FosB levels in the striatum but not in the prefrontal cortex, suggesting the EE effects on  $\Delta$ FosB are selective for this brain region. Finally, our study highlights and confirms the importance of investigation of cell-type specificity to better understand the consequences of different manipulations on brain neuroadaptations.

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## Statement of Interest

None.

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