Supporting Information

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SI Text

Detailed Statistical Analysis for c-FOS Results. A three-way ANOVA was used to analyze c-FOS results with environment (enriched or standard), conditioning (saline or cocaine), and challenge (saline or cocaine) as factors.

Statistical analysis revealed that environmental enrichment significantly decrease the ability of cocaine to activate 5 of the 12 brain regions sampled, namely the infralimbic cortex, the shell and the core of the nucleus accumbens, the ventral tegmental area, and the basolateral amygdala (**infralimbic cortex**: Environment effect, $F_{1,44} = 9.16$, $P = 0.0042$; Conditioning effect, $F_{1,44} = 25.84$, < 0.0001; Challenge effect, $F_{1,44} = 70.91$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 2.04$, $P = 0.16$; Environment × Challenge interaction, $F_{1,44} = 1.00$, $P = 0.32$; Conditioning × Challenge interaction, $F_{1,44} = 12.59$, $P = 0.0010$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 1.57$, $P = 0.22$; **shell**: Environment effect, $F_{1,44} = 10.93$, $P = 0.0019$; Conditioning effect, $F_{1,44} = 32.16$, $P < 0.0001$; Challenge effect, $F_{1,44} = 40.99$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 13.29$, $P = 0.0007$; Environment × Challenge interaction, $F_{1,44} = 13.17$, $P = 0.0007$; Conditioning × Challenge interaction, $F_{1,44} = 19.94$, $P < 0.0001$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 8.80$, $P = 0.0048$; **core**: Environment effect, $F_{1,44} = 4.04$, $P = 0.050$; Conditioning effect, $F_{1,44} = 54.59$, $P < 0.0001$; Challenge effect, $F_{1,44} = 49.82$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 3.55$, $P = 0.067$; Environment × Challenge interaction, $F_{1,44} = 3.27$, $P = 0.077$; Conditioning × Challenge interaction, $F_{1,44} = 39.35$, $P < 0.0001$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 3.30$, $P = 0.076$; **VTA**: Environment effect, $F_{1,44} = 30.16$, $P < 0.0001$; Conditioning effect, $F_{1,44} = 29.42$, $P < 0.0001$; Challenge effect, $F_{1,44} = 117.42$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 27.52$, $P < 0.0001$; Environment × Challenge interaction, $F_{1,44} = 26.37$, $P < 0.0001$; Conditioning × Challenge interaction, $F_{1,44} = 20.62$, $P < 0.0001$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 21.79$, $P < 0.0001$; **BLA**: Environment effect, $F_{1,44} = 2.77$, $P = 0.103$; Conditioning effect, $F_{1,44} = 23.14$, $P < 0.0001$; Challenge effect, $F_{1,44} = 28.43$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 5.21$, $P = 0.027$; Environment × Challenge interaction, $F_{1,44} = 0.\ 76$, $P < 0.38$; Conditioning × Challenge interaction, $F_{1,44} = 9.57$, $P = 0.0035$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 3.32$, $P < 0.075$).

In 6 of the 12 brain regions sampled (prelimbic cortex, anterior cingulate cortex, orbitofrontal cortex, central amygdala, ventral pallidum, and dorsolateral caudate putamen), cocaine challenge produced similar activation in enriched and standard mice conditioned to cocaine (**prelimbic cortex**: Environment effect, $F_{1,44} = 3.46$, $P = 0.069$; Conditioning effect, $F_{1,44} = 25.22$, $P < 0.0001$; Challenge effect, $F_{1,44} = 27.60$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 1.70$, $P = 0.199$; Environment × Challenge interaction, $F_{1,44} = 0.021$, $P = 0.89$; Conditioning × Challenge interaction, $F_{1,44} = 36.31$, $P < 0.0001$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 0.49$, $P = 0.49$; **anterior cingulate cortex**: Environment effect, $F_{1,44} = 0.054$, $P = 0.82$; Conditioning effect, $F_{1,44} = 11.37$, $P = 0.016$; Challenge effect, $F_{1,44} = 19.12$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 0.009$, $P = 0.93$; Environment × Challenge interaction, $F_{1,44} = 0.42$, $P = 0.89$; Conditioning × Challenge interaction, $F_{1,44} = 17.24$, $P = 0.0001$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 0.55$, $P = 0.46$; **orbitofrontal cortex**: Environment effect, $F_{1,44} = 2.17$, $P = 0.15$; Conditioning effect, $F_{1,44} = 1.16$, $P = 0.29$; Challenge effect, $F_{1,44} = 46.10$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 0.45$, $P = 0.51$; Environment × Challenge interaction, $F_{1,44} = 0.86$, $P = 0.36$; Conditioning × Challenge interaction, $F_{1,44} = 0.15$, $P = 0.70$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 0.01$, $P = 0.93$; **central amygdala**: Environment effect, $F_{1,44} = 0.82$, $P = 0.77$; Conditioning effect, $F_{1,44} = 4.08$, $P = 0.049$; Challenge effect, $F_{1,44} = 12.03$, $P = 0.0012$; Environment × Conditioning interaction, $F_{1,44} = 0.51$, $P = 0.48$; Environment × Challenge interaction, $F_{1,44} = 0.064$, $P = 0.80$; Conditioning × Challenge interaction, $F_{1,44} = 14.64$, $P = 0.0004$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 0.019$, $P = 0.89$; **ventral pallidum**: Environment effect, $F_{1,44} = 2.53$, $P = 0.12$; Conditioning effect, $F_{1,44} = 32.96$, $P < 0.0001$; Challenge effect, $F_{1,44} = 17.61$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 1.01$, $P = 0.32$; Environment × Challenge interaction, $F_{1,44} = 0.019$, $P = 0.89$; Conditioning × Challenge interaction, $F_{1,44} = 27.21$, $P < 0.0001$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 1.64$, $P = 0.21$; **dorsolateral caudate putamen**: Environment effect, $F_{1,44} = 0.84$, $P = 0.36$; Conditioning effect, $F_{1,44} = 19.97$, $P < 0.0001$; Challenge effect, $F_{1,44} = 22.18$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 1.56$, $P = 0.22$; Environment × Challenge interaction, $F_{1,44} = 1.17$, $P = 0.28$; Conditioning × Challenge interaction, $F_{1,44} = 25.83$, $P < 0.0001$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 0.81$, $P = 0.37$).

In the dentate gyrus of the hippocampus, environmental enrichment increased the number of c-FOS-positive nuclei compared with standard conditions. In this region cocaine increased c-FOS-positive nuclei independently of environmental condition (**dentate gyrus**: Environment effect, $F_{1,44} = 32.49$, $P < 0.0001$; Conditioning effect, $F_{1,44} = 0.87$, $P = 0.35$; Challenge effect, $F_{1,44} = 23.29$, $P < 0.0001$; Environment × Conditioning interaction, $F_{1,44} = 0.018$, $P = 0.89$; Environment × Challenge interaction, $F_{1,44} = 0.48$, $P = 0.49$; Conditioning × Challenge interaction, $F_{1,44} = 1.79$, $P = 0.19$; Environment × Conditioning × Challenge interaction, $F_{1,44} = 0.61$, $P = 0.61$).

SI Materials and Methods

Experiment 1: Behavioral Sensitization. Motor activity was measured in Plexiglas cages (19 × 11 × 14 cm) placed in frames mounted with computer-monitored photocell beams (Imetronic). Horizontal locomotion was measured by the number of cage crossings. Mice were first habituated to locomotor chambers for 60 min. Then they were injected i.p. with drug or saline and immediately placed back in the chamber, and locomotor activity was measured for 90 min.

Behavioral sensitization consisted of two phases: development and expression. For development of behavioral sensitization 6 injections of cocaine (15 mg/kg i.p.) or saline were administered every second day. During this phase all mice were housed in standard environments (SE). At the end of the last sensitization session, half the mice were kept in SE and the other half were switched to enriched environments (EE). All mice stayed in the respective environmental housing condition in the animal facility until the day of testing for expression of behavioral sensitization. Expression of behavioral sensitization (i.e., response to a challenge injection of 10 mg/kg of cocaine) was measured 1, 7, or 30 days after the last injection of cocaine in separate groups of mice. Control groups of mice, housed in SE, were administered six

times with saline injections then separated in either SE or EE and challenged with 10 mg/kg of cocaine 30 days after the last saline injection. For a graphic representation of the procedures of experiment 1, see Fig. 1*a*.

Two supplementary groups of mice ($n = 11$ per group) were used to test the possibility that social isolation could also alter already-established behavioral sensitization. These mice were sensitized to cocaine as described above and, after development of behavioral sensitization, were either assigned to SE or isolated environment (IE) conditions. IE consisted of small housing cages ($20 \times 10 \times 12$) made of opaque Plexiglas in which mice were housed one per cage. Mice were kept in SE or IE for 30 days in the animal facility before being tested for expression of behavioral sensitization.

General Procedure for Cocaine-Conditioned Place Preference. Conditioned place preference experiments were performed in four identical computer-monitored boxes (Imetronic) formed by two lateral chambers ($15 \times 15 \times 20$ cm) connected by a central alley ($5 \times 15 \times 20$ cm). Two sliding doors separated the alley from the chambers. In each chamber, two Plexiglas prisms with triangular bases ($5 \times 7 \times 19$ cm) were arranged to form different patterns (always covering the same surface of the chamber) and were used as conditioned stimuli. Two different metallic grids on the floor, one with large (1 cm) squares and the other with small (0.5 cm) circles, were also used as conditioned stimuli. Two infrared photocells were present in each compartment and detected the presence and movements of mice. General conditioned place preference procedure consisted of three phases: preconditioning, conditioning, and test. For each manipulation mice were brought to the experimental room 60 min before the start of the experiment to allow for habituation and to reduce stress. For preconditioning, mice were placed in the central alley with the doors closed. After 15 sec the doors were opened and mice were free to explore the entire two-compartment apparatus for 30 min. The time spent in each compartment was recorded and was considered a measure of spontaneous preference. Conditioning sessions were performed on the following 4 days, twice per day with morning and afternoon sessions separated by at least 6 h. During these sessions, mice were injected with either saline or cocaine (10 mg/kg) and immediately confined to one of the pairing compartments for 30 min. The order of treatments (saline or cocaine), the time of cocaine injection (morning or afternoon), and the compartment (right or left) were counterbalanced. A total of four cocaine and four saline conditioning sessions was performed. Test sessions for expression of conditioned place preference were similar to preconditioning sessions, with animals placed in the central alley for 15 sec and then left free to choose a compartment for 30 min. The time spent in each compartment was measured and compared with the time spent in the same compartment during the preconditioning session. Preference scores, which served as a measure of rewarding effects of cocaine, were calculated by subtracting the time in seconds spent during the test from the time spent during the pretest in the compartment paired to cocaine injections. Procedures for the development of conditioned place preference (preconditioning and conditioning) were performed similarly for both experiment 2 (expression of conditioned place preference) and experiment 3 (extinction and reinstatement of conditioned place preference). During this phase all mice were housed in SE. In contrast, postconditioning procedures differed between experiment 2 and 3 as detailed next.

Procedure for Lithium-Induced Conditioned Place Aversion. Mice were conditioned to lithium chloride (3 mEq/kg) in the same place conditioning apparatus and with a procedure similar to that previously described. For preconditioning, mice were placed in the central alley with the doors closed and left free to explore the entire two-compartment apparatus for 30 min. To avoid carryover effects of lithium injections, conditioning sessions were performed once per day for the following 8 days. During these sessions, mice were injected with either saline or lithium (3 mEq/kg) and immediately confined to one of the pairing compartments for 30 min. The order of treatments (saline or lithium), the time of cocaine injection (morning or afternoon), and the compartment (right or left) were counterbalanced. A total of four lithium and four saline conditioning sessions was performed. Test sessions for expression of conditioned place aversion were similar to preconditioning sessions, with animals placed in the central alley for 15 sec and then left free to choose a compartment for 30 min.

Experiment 2a: Expression of Cocaine-Conditioned Place Preference. At the end of the last conditioning session, half the mice were kept in SE and the other half were switched to EE. All mice stayed in the respective environmental housing condition in the animal facility until the day of testing for expression of conditioned place preferences. Test sessions in drug-free states were conducted 1, 7, or 30 days after the last conditioning session, and preference scores were obtained as detailed above. For a graphic representation of the procedures of experiment 2, see Fig. 1*b*.

Experiment 3: Extinction and Reinstatement of Conditioned Place Preference. The day after the last conditioning sessions, a test session for conditioned place preference was conducted, and preference scores were obtained as detailed above. Control mice received only saline during conditioning sessions, and preference scores were calculated arbitrarily using the time spent in the left compartment, as we have previously done (1). As expected, this group developed no place preference. For mice conditioned to cocaine, only mice that showed a preference for the cocaine-paired compartment were included in the study. At the end of this test session, half the mice were kept in SE and the other half were switched to EE. The next day and for 10 consecutive days, conditioned place preferences were extinguished by running 30-min test sessions similar to the previous one. On the 11th day, mice were injected with either saline or 10 mg/kg of cocaine, and reinstatement of extinguished conditioned place preferences was studied. The reinstatement session was similar to the previous ones. A total of eight groups was obtained: SE sal-sal ($n = 6$), sal-coc ($n = 8$), coc-sal ($n = 6$), and coc-coc ($n = 12$) and EE sal-sal ($n = 6$), sal-coc ($n = 8$), coc-sal ($n = 6$), and coc-coc ($n = 12$). Sal-sal mice received saline both during conditioning and reinstatement of conditioned place preference; sal-coc mice received saline during conditioning and cocaine for reinstatement of conditioned place preference; coc-sal mice received cocaine during conditioning and saline for reinstatement of conditioned place preference; coc-coc mice received cocaine both during conditioning and reinstatement of conditioned place preference. Reinstatement scores were calculated by subtracting the time in seconds spent during the reinstatement session from the time spent during the last extinction session in the compartment paired to cocaine injections. Locomotor counts were monitored during the reinstatement session Fig. S1. For a graphic representation of the procedures of experiment 3, see Fig. 1*c*.

Brain Tissue Preparation and Immunohistochemistry. Fifty-two mice from experiment 3 ($n = 6$–8 per group) were used for immunohistochemistry analysis. The same eight experimental groups were analyzed: SE sal-sal, sal-coc, coc-sal, and coc-coc and EE sal-sal, sal-coc, coc-sal, and coc-coc. Ninety min after behavioral testing (≈120 min after saline or cocaine injection), mice were deeply anesthetized using 400 mg/kg i.p. of chloral hydrate (Sigma-Aldrich) and intracardially perfused with 0.9% NaCl followed by 4% paraformaldehyde (PFA) dissolved in 0.1 M

phosphate buffer (pH 7.4). Brains were then removed and postfixed in 4% PFA for 1 h and stored in 30% sucrose/4% PFA at 4°C until sectioning. All serial brain sections (40 μm) were then cut using a freezing microtome (Leica RM2145). Sections were stored in cryoprotective solution (glycerol 20%, DMSO 2%, NaCl 0.9%, phosphate buffer 0.1 M) at −20°C until processed for immunolabeling.

Free floating sections from mice in different groups were processed simultaneously for c-FOS protein expression. Sections were washed extensively in 0.1 M PBS (three times for 10 min each) and incubated for 30 min in 0.3% hydrogen peroxidase. Then they were washed extensively in 0.1 M PBS (three times for 10 min each) and incubated for 2 h in 0.3% Triton X-100 in 0.1 M PBS containing 3% BSA (Sigma-Aldrich). Subsequently sections were incubated for 48 h at 4°C with the anti-c-FOS rabbit polyclonal primary antibody (1:10,000, Sigma) containing 0.1 M PBS, 0.3% Triton X-100, and 3% BSA. Then sections were washed in 0.1 M PBS (three times for 10 min each) and incubated for 2 h in 0.1 M PBS containing biotinylated goat antirabbit antibody IgG (1:600, Vector Laboratories), 0.3% Triton X-100, and 3% BSA. Afterward the tissue was given additional washes in 0.1 M PBS (three times for 10 min each) and incubated for 2 h in avidin-biotinylated horseradish peroxidase complex (ABC Elite kit, Vector Laboratories) diluted in 0.1 M PBS. Then, sections were washed twice in 0.1 M PBS, followed by a wash in 0.05 M Tris buffer (pH 7.6), and they were incubated in 0.025% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich) containing 0.08% nickel ammonium sulfate (Sigma) and 0.01% hydrogen peroxidase (Sigma-Aldrich) for 3–5 min. This reaction was terminated by rinsing the tissue in 0.1 M PBS (two times for 10 min each) and then 0.1 M Tris buffer (10 min). Finally, sections were then mounted onto gelatin-coated slides, dried, and dehydrated before coverslipping.

Immunoreactivity Analysis. c-FOS-specific immunoreactivity was examined using an Olympus optical microscope set at ×40 magnification and counted by an observer blind to treatment as previously described (2). Fig. S2 illustrates the specific subregions analyzed. Sections taken at +1.98 mm from bregma contained (*i*) prelimbic, (*ii*) infralimbic, and (*iii*) orbitofrontal cortex; sections taken at +1.18 mm from bregma contained the (*iv*) anterior cingulate cortex, (*v*) the dorsolateral caudate putamen, and (*vi*) the shell and (*vii*) core of the nucleus accumbens; sections taken at +0.14 mm from bregma contained (*viii*) the ventral pallidum; sections taken at −1.7 mm from bregma contained (*ix*) the dentate gyrus of the hippocampus and (*x*) the central and (*xi*) basolateral amygdala; sections taken at −3.08 mm from bregma contained (*xii*) the ventral tegmental area. The number of immunoreactive cells in each region was counted from each hemisphere of three sections labeled for c-FOS. The counts from all of the sample areas of a given region were averaged to a mean number of immunoreactive cells per area per mouse.

1. Solinas M, Thiriet N, Rawas RE, Lardeux V, Jaber (May 7, 2008) Environmental enrichment during early stages of life reduces the behavioral, neurochemical, and molecular effects of cocaine. *Neuropsychopharmacology*, 10.1038/npp.2008.51.

2. Neisewander JL, *et al.* (2000) Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. *J Neurosci* 20:798–805.

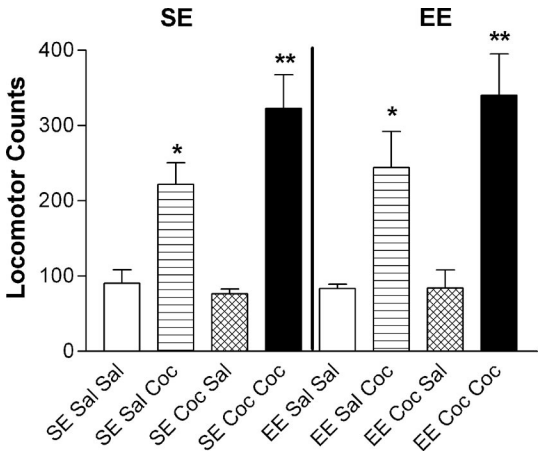

Fig. S1. Locomotor activity during reinstatement test of experiment 3. Locomotor counts in the conditioned place preferences apparatus were assessed after injection of saline or cocaine (10 mg/kg) challenge in mice housed in SE (*Left*) or EE (*Right*) environments after development of conditioned place preferences and during extinction. Note that cocaine increases locomotor activity regardless of previous exposure to cocaine or environmental condition (three-way ANOVA: Challenge effect, $F_{1,56} = 37.57$, $P < 0.0001$). On the one hand, the fact that cocaine-induced increases in locomotion activity did not differ in cocaine-treated and cocaine-naïve mice indicates that, in our conditioned place preference procedure, mice do not develop significant behavioral sensitization. On the other hand, the fact that cocaine-induced increases in locomotion activity did not differ in SE and EE mice indicates (*i*) that behavioral activation and cocaine seeking are independent phenomena and (*ii*) that motor behavior cannot account for the differences in c-FOS expression found between SE and EE. Whereas these results may seem to contrast with those in experiment 1 (Fig. 2*b*), they do not. Indeed, in experiment 1, 7 days of environmental enrichment were not sufficient to eliminate behavioral sensitization; thus, it is not surprising that in experiment 3, 10 days of environmental enrichment may also not be sufficient to affect cocaine-induced locomotion. In addition, the lack of significant sensitization to cocaine, as well the many procedural differences (number of injections, dose, frequency of injection, time spent in enriched environments), can also explain why, in contrast to experiment 1, SE and EE mice show similar cocaine-induced locomotion during the reinstatement test. Results represent the means ± SEM from 6–12 mice. Three-way ANOVA followed by *post hoc* Student-Newman-Keuls's test: $^{**}P < 0.01$ vs. respective challenge control.

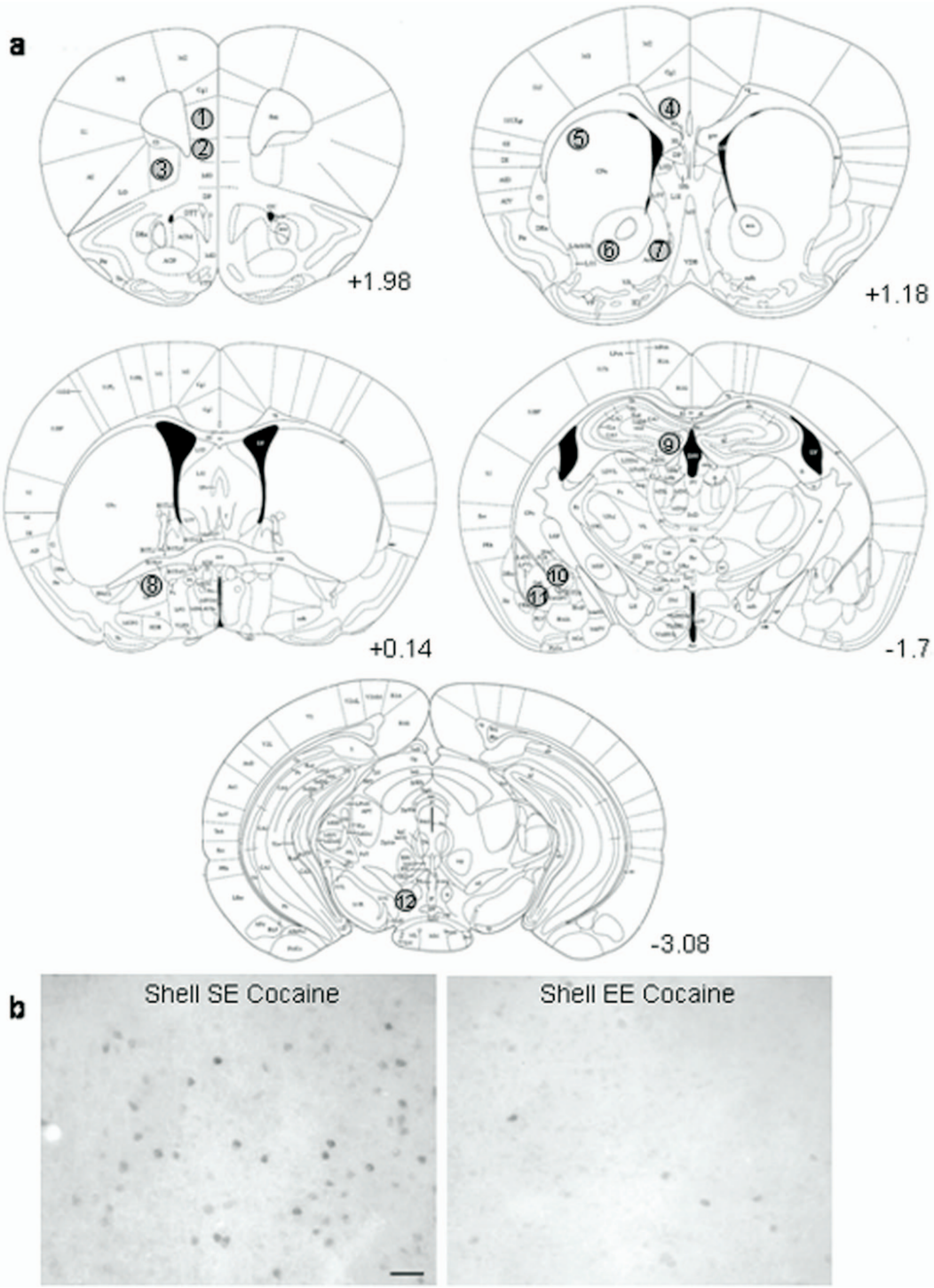

Fig. S2. Schematic representation of the brain regions that were quantified for expression of c-FOS by immunohistochemistry. Numbers beside each plate represent distance from bregma. (*a*) Sections taken at +1.98 mm from bregma contained (*1*) prelimbic, (*2*) infralimbic, and (*3*) orbitofrontal cortex; sections taken at +1.18 mm from bregma contained the (*4*) anterior cingulate cortex, (*5*) the dorsolateral caudate putamen, and (*6*) the shell and (*7*) core of the nucleus accumbens; sections taken at +0.14 mm from bregma contained (*8*) the ventral pallidum; sections taken at −1.7 mm from bregma contained (*9*) the dentate gyrus of the hippocampus and (*10*) the central and (*11*) basolateral amygdala; sections taken at −3.08 mm from bregma contained (*12*) the ventral tegmental area. Drawings are modified from the atlas Paxinos and Franklin [Paxinos G, Franklin KBJ (2001) *The Mouse Brain in Stereotoxic Coordinates* (Academic Press, San Diego), 2nd Ed.]. (*b*) Representative photomicrograph illustrating quantitative data shown in Fig. 5. Slides show FOS-immunoreactive nuclei within the shell of the nucleus accumbens in (*Left*) one mouse housed in SE and (*Right*) one mouse housed in EE injected with 10 mg/kg of cocaine immediately before the reinstatement test session. (Scale bar, 30 μm.)

Table S1. FOS-positive nuclei in all regions sampled

	SE				EE			
Brain region	Sal-Sal ($n = 6$)	Sal-Coc ($n = 8$)	Coc-Sal ($n = 6$)	Coc-Coc ($n = 6$)	Sal-Sal ($n = 6$)	Sal-Coc ($n = 8$)	Coc-Sal ($n = 6$)	Coc-Coc ($n = 6$)
Nucleus accumbens shell	10.20 ± 1.31	14.56 ± 1.21	13.36 ± 1.49	42.72 ± 5.30†	11.87 ± 1.54	14.02 ± 2.18	12.76 ± 0.98	19.44 ± 2.97§
Nucleus accumbens core	6.62 ± 1.59	7.51 ± 0.83	7.97 ± 1.10	27.29 ± 2.29†	6.47 ± 1.42	7.37 ± 1.06	7.67 ± 1.41	18.72 ± 2.82†§
Dorsolateral caudate putamen	4.90 ± 0.61	4.77 ± 0.55	4.72 ± 0.45	12.72 ± 2.26†	5.25 ± 0.53	4.87 ± 0.77	4.60 ± 0.35	9.90 ± 0.96†
Ventral tegmental area	4.25 ± 0.69	9.79 ± 0.95	5.44 ± 0.72	26.04 ± 2.31†	4.43 ± 0.80	9.21 ± 1.36	4.68 ± 0.43	9.25 ± 0.46§
Basolateral amygdala	12.50 ± 1.07	14.14 ± 1.77	15.28 ± 2.09	29.46 ± 2.07†	12.08 ± 1.34	16.14 ± 1.84	13.68 ± 1.28	20.99 ± 2.35‡
Central amygdala	8.33 ± 2.98	8.35 ± 1.89	7.75 ± 2.56	12.28 ± 2.98*	8.83 ± 0.75	8.37 ± 1.92	7.20 ± 1.04	11.60 ± 2.92*
Ventral pallidum	7..33 ± 0.61	5.87 ± 0.67	7.50 ± 0.73	14.80 ± 1.20†	6.17 ± 0.65	6.25 ± 0.92	6.71 ± 0.43	12.10 ± 1.78†
Hippocampal dentate gyrus	6.87 ± 0.81	9.72 ± 1.49	7.08 ± 0.48	11.34 ± 0.80	11.74 ± 1.06	14.89 ± 1.87	10.83 ± 0.86	17.17 ± 0.95*‡
Prelimbic cortex	23.17 ± 1.49	21.37 ± 1.28	22.62 ± 2.26	38.97 ± 2.71†	21.67 ± 1.84	21.37 ± 0.96	19.50 ± 1.71	33.57 ± 2.87†
Anterior cingulate cortex	26.17 ± 2.55	26.37 ± 1.22	23.58 ± 1.60	39.47 ± 3.39†	25.50 ± 1.94	26.00 ± 1.18	25.58 ± 1.96	37.02 ± 3.83†
Infralimbic cortex	15.4 ± 1.2	18.89 ± 0.70	16.69 ± 0.90	27.45 ± 1.75†	14.00 ± 0.93	17.87 ± 0.95	15.03 ± 0.64	22.37 ± 1.14†§
Orbitofrontal cortex	11.80 ± 1.32	20.74 ± 1.34*	13.36 ± 2.05	23.49 ± 3.17†	11.83 ± 1.74	18.71 ± 0.68*	11.97 ± 0.83	19.58 ± 1.75*

Values are mean ± SEM. *, $P < 0.05$; †, $P < 0.01$ vs. saline challenge control. ‡, $P < 0.05$; §, $P < 0.01$ vs. standard environment control.