Supporting Information

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SI Text

Materials and Methods

All procedures involving the use of the animals and analysis of brain anatomy were approved by the Institutional Care and Use Committees of Fujita Health University and Massachusetts Institute of Technology, and the behavioral analyses performed were approved by the ethical guidelines of Fujita Health University.

Animals and Motor Behavioral Tests. Mice were generated and genotyped as described elsewhere (1). All tests were performed by investigators blind to the genotypes of the mice. For the test of hindlimb clasping, the animal groups comprised of both DPS and wildtype mice at the ages of 12–13 wk (adult; DPS, $n = 10$; wildtype, $n = 10$) and 2 wk (DPS, $n = 12$; wildtype, $n = 53$). Each mouse was picked up by its tail and suspended for 15 s to observe hindlimb clasping (2). On the beam-walking test (3), adult DPS ($n = 10$) and wildtype ($n = 10$) mice 12–13 wk old were trained to traverse a medium square beam (12 mm wide) in three consecutive trials each day for 2 days. After training, animals were tested with two trials per day for their beam transversal time and the number of hind paw slips to cross square beams (6 mm wide) and the round beams (14 mm and 6 mm diameter). The data from the beam-walking test were analyzed by two-way ANOVA with repeated measurement; P values of less than 0.05 were considered to be statistically significant.

Apomorphine Injections. In two wild-type and two DPS mice, apomorphine (Sigma) dissolved in 0.1% ascorbic acid was administered i.p. at a dose of 5 mg/kg. In controls, 0.9% NaCl was injected i.p. Animals were perfused transcardially (4% paraformaldehyde in 0.1 M PB) 2 h after the injections. For double immunofluorescence staining, primary antibodies to FosB (Santa Cruz Biotechnology; 1:2,000), TH (Chemicon International; 1:1,000) and MOR were used with secondary antibodies labeled by Alexa Fluor 488 and Alexa Fluor 568 (Invitrogen; 1:1,000). For each animal, counts of FosB-positive nuclei were made in five sections at mid-anteroposterior levels of the caudoputamen to estimate the density of FosB-positive nuclei in the matrix and in striosomes.

Tissue Preparation. Mice were given an i.p. injection of a lethal dose of pentobarbital and were perfused transcardially with 0.9% saline in 0.01 M phosphate buffer, pH 7.4 (PBS), followed by cold 0.1 M phosphate buffer (PB, pH 7.4) containing 4% paraformaldehyde. The brains were removed, were postfixed overnight in the same fixative at 4°C, and were stored in 0.1 M PB containing gradient (10 to 30%) sucrose at 4°C for cryoprotection. Sections were cut on a cryostat at 30 μm and were stored in PBS 0.001% NaN_3 until use.

Immunohistochemistry. Immunohistochemical staining was performed on free-floating sections (4) with antisera raised against TH (5) (1:100,000), AADC (39) (1:20,000), and MOR (Oncogene Science; 1:10,000). For detection of the bound antibodies, we used the Elite ABC kit (Vector Laboratory), usually with the Histofine Simple Stain kit (Nichirei Co.), with diaminobenzidine (DAB) as the chromogen.

Digital Imaging and Densitometry. The immunostained sections were studied microscopically. Digital images were acquired with MetaMorph software (Molecular Devices), imported into Adobe Photoshop Elements 2.0 and processed digitally. To estimate the density of TH labeling, we immunostained in parallel, at the same time, striatal sections from both DPS and wild-type mice at the age of 1–2 days (0 wk; $n = 6$), 1 wk ($n = 6$), 2 wk ($n = 5$), 4 wk ($n = 5$), and 12–13 wk (adult; $n = 6$). The optical densities of DAB products were measured as gray levels. For each animal, measurements were made in five sections per anterioposterior level (anterior, middle, and posterior; see Fig. 3). When striosomes were not clearly visible, they were identified by comparison with the adjacent sections stained for MOR. For statistical analysis we used ANOVA with adjusted multiple comparisons, with P values of less than 0.05 considered as statistically significant.

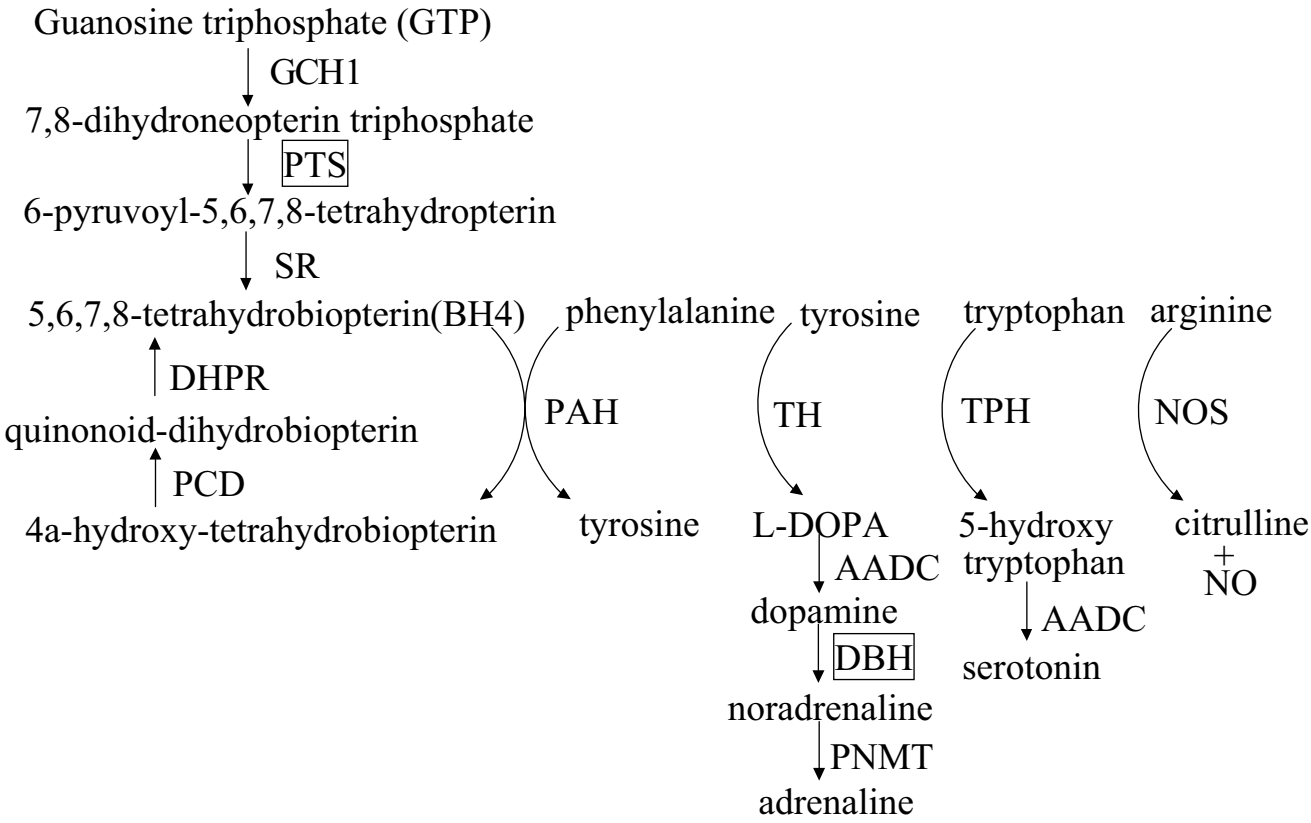

Fig. S1. Biosynthetic pathway of BH4 and monoamine biosynthesis. Enzymes are indicated by the following abbreviations: GCH1, GTP cyclohydrolase I; PTS, 6-pyruvoyltetrahydropterin synthase; SR, sepiapterin reductase; DHPR, dihydropteridine reductase; PCD, pterin-4-acarbinolamine dehydratase; PAH, phenylalanine hydroxylase; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; NOS, nitric oxide synthase; AADC, aromatic L-amino acid decarboxylase; DBH, dopamine β-hydroxylase; PNMT, phenylethanolamine N-methyl transferase. PTS, the gene targeted in the model mouse studied here, and DBH are boxed.

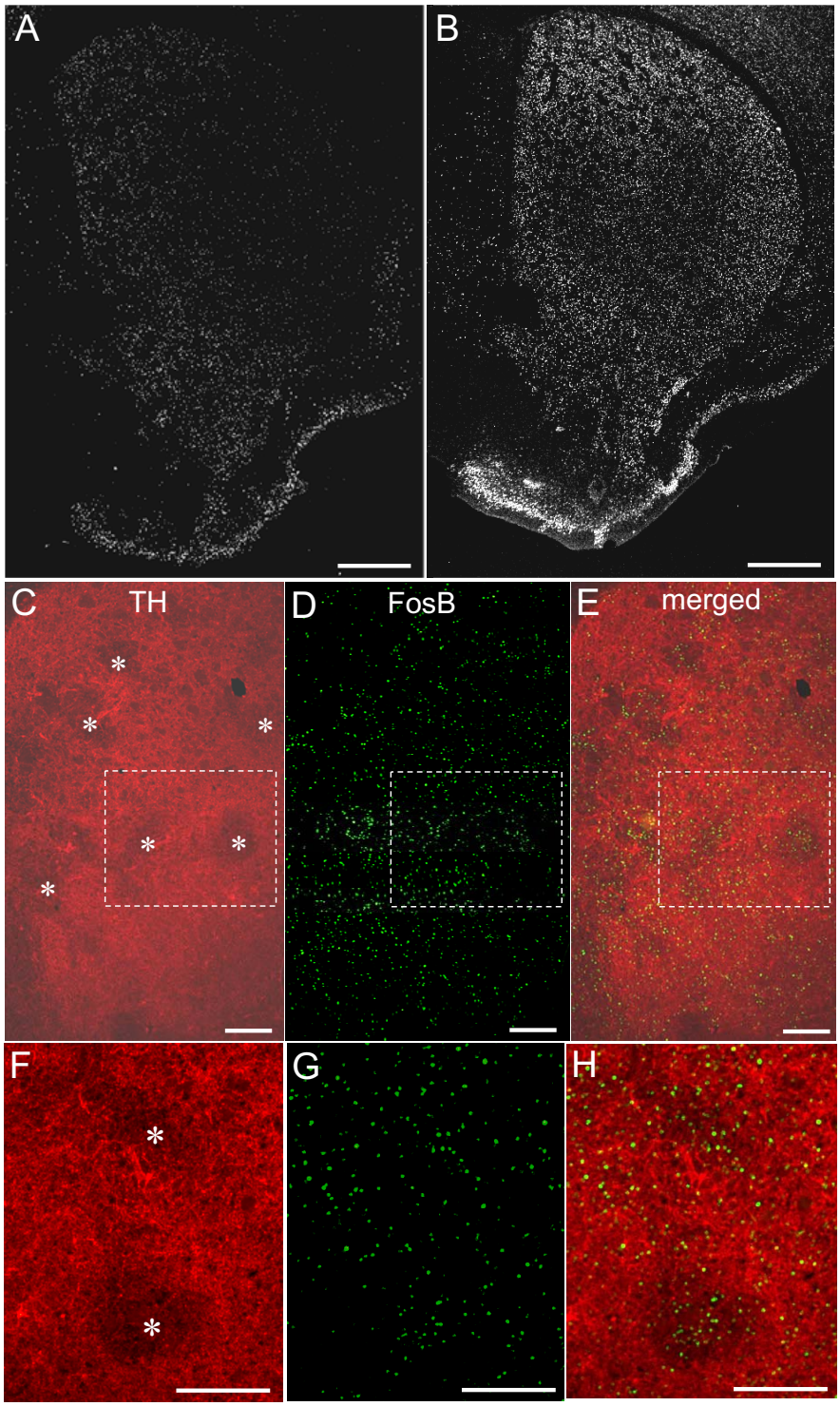

Fig. S2. Striatal FosB induction after apomorphine treatment in a DPS mouse. (*A* and *B*) Negative prints of FosB-labeled cross-sections of dorsal striatum from a wildtype (*A*) and a DPS mouse after apomorphine administration (5 mg/kg, i.p.). (*C–E*) Double-immunostaining of a striatal cross-section with antibodies to TH (red) and FosB (green) from a DPS mouse given apomorphine treatment. Arrows mark examples of TH-poor zones (i.e., striosomes). (*F–H*) Higher-power illustrations of the striatal regions indicated by hatched boxes in *C–E*. Asterisks mark TH-poor zones. Scale bars: *A* and B, 500 μm; and *C-H*, 200 μm.

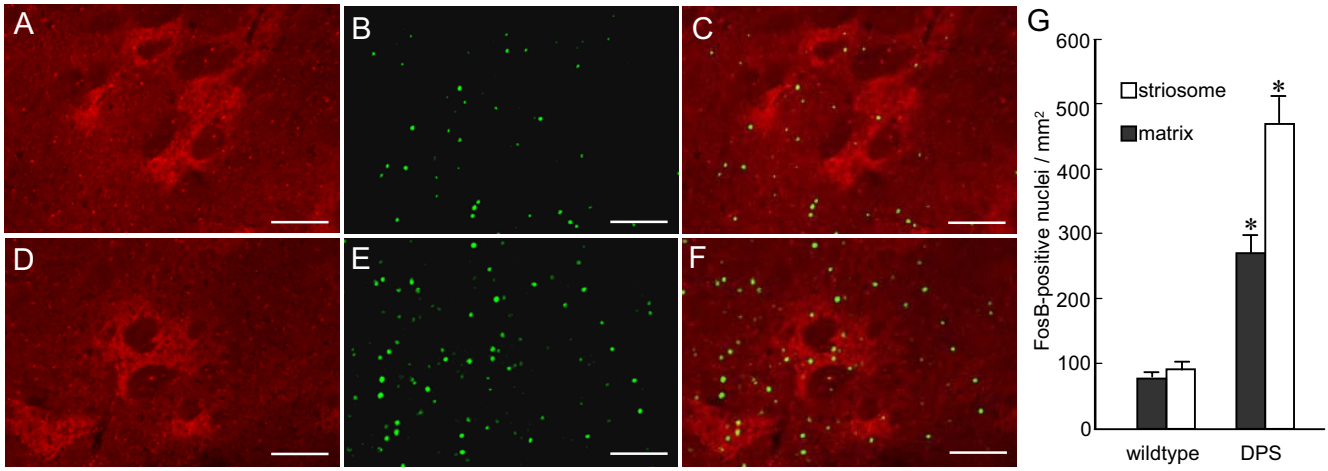

Fig. S3. FosB induction in striosome and matrix compartments after apomorphine treatment. (*A–F*) Double-immunofluorescence staining of striatal cross-sections with antibodies to MOR (red) and FosB (green) from wildtype (*A–C*) and DPS (*D–F*) mice after apomorphine administration. (*G*) Density of nuclei immunopositive for FosB in the matrix (black) and striosomes (white) from wild-type and DPS mice treated with apomorphine administration. Values indicate numbers of immunopositive nuclei/mm^2 (means ± SEMs). * indicates $P < 0.05$. Scale bars: *A–F*, 100 μm.

Movie S1. ●●●.

Movie S1 (WMV)

Movie S2. ●●●.

Movie S2 (WMV)