SUPPLEMENTAL METHODS

Transgenic mouse systems.

All bitransgenic mice were conceived and raised on 100 µg doxycycline (Sigma) to suppress transgene expression during development as described\textsuperscript{21, 23, 25}. At 8 wks of age, half of the littermates remained on doxycycline and half were switched to water and experiments were performed 8 wks later when the transcriptional effects of ∆FosB are maximal\textsuperscript{14, 23}. Equivalent transgene expression is seen in the core and shell subregions of NAc in all three mouse lines\textsuperscript{21, 23, 25}. Use of animals was approved by UT Southwestern’s IACUC (protocol 08930301-1).

AAV-mediated gene transfer.

Stereotaxic coordinates for AAV vector injections into the NAc were: anterior-posterior +1.5 mm, lateral ± 1.5 mm, and dorsoventral -4.4 mm at an angle of 10° from the midline (relative to Bregma). The core and shell subregions of NAc are affected equally by these injections.

Western blotting.

We used the following antisera for western blot analysis: Gαi\textsubscript{1} (1:1000), Gαi\textsubscript{2} (1:2000), Gβ (1:10,000), and Gs (1:2000), all provided by Suzanne Mumby (UT Southwestern Medical Center), RGS9-2 (1:2000) provided by Stephen Gold (UT Southwestern Medical Center), Gβ5 (1:10,000) provided by William Simonds (NIDDK),
Gq (1:2000, Chemicon), and spinophilin (1:10,000) provided by Patrick Allen (Yale University).

Opioid receptor binding assays.

NAc was dissected on ice, and membranes were prepared by homogenizing tissue in 50 mM Tri-HCl, pH 7.4, 3 mM MgCl₂ and 1 mM EGTA, centrifuging for 10 min (4°C) at 50,000 x g, discarding the supernatant, resuspending the pellet and repeating the centrifugation. Membranes were resuspended in 50 mM Tris-HCl, 100 mM NaCl, 3 mM MgCl₂ and 0.2 mM EGTA (assay buffer) and pretreated with adenosine deaminase as described (see references in Supplemental Information). Membranes (75-100 µg protein, pooled from 2-3 mice) were incubated for 90 min at 30°C in assay buffer with 2 nM [³H]naloxone, 0.5 nM unlabeled naltrindole, and 2 nM unlabeled Nor-BNI, which provides a specific measure of µ receptors. Membranes were incubated with [³H]DADLE or [³H]CTAP to provide a specific measure of δ or µ receptors, respectively. Nonspecific binding was determined with 10 µM unlabeled naltrexone. The incubation was terminated by vacuum filtration. Bound radioactivity was determined by liquid scintillation spectrophotometry at 45% efficiency for [³H]. See supplemental references for further details.

Chromatin immunoprecipitation assays.

Chromatin immunoprecipitation assays were performed exactly according to published methods (see Supplemental References). Briefly, punch dissections of NAc were treated with 1% formaldehyde for 15 min at room temperature to cross-link DNA
and its associated proteins, and then washed 5 times with cold PBS. After incubation with specific antisera, immunoprecipitations were accomplished by use of Protein A agarose beads in a salmon sperm DNA slurry. The immunoprecipitates were washed stringently and reverse cross-linked with NaCl at 65°C, the immunoprecipitated DNA was purified using phenol chloroform and ethanol precipitation, and quantified using real time PCR. The binding of ∆FosB to the dynorphin promoter was determined by measuring the amount of the dynorphin promoter pulled down in chromatin immunoprecipitates by use of real-time PCR (ABI Prism 7700, Applied Biosystems) using oligonucleotide pairs designed to amplify proximal (~100-200 bp long) promoter regions of dynorphin and of synaptotagmin (analyzed as a control). The oligonucleotides used were 5’- CGCTTCTCTGTGGCACTTC-3’ and 5’- TTGTCCCTGGCAGGCTTCTG-3’ for prodynorphin; 5’-TAGAACCTCTGCGGTCGT-3’ and 5’-TCATCTGGTAGAAGTGTCGAGGAGA-3’ for synaptophysin. Input and immunoprecipitated DNA were PCR amplified in triplicate in the presence of SYBR-Green (ABI). Ct values from each sample were obtained using the Sequence Detector 1.1 software. Relative quantification of amplified template was performed as described in detail in Supplemental References.

**Statistical analysis.**

For place preference, analgesia, and opiate withdrawal behavioral assays, we used two way ANOVAs and Bonferroni post hoc tests for within groups comparisons whenever analysis revealed a genotype affect. For receptor binding,
immunohistochemistry, western blotting, and chromatin immunoprecipitation assays, we used t-tests.