Supplementary Data 1.

Animal experiments (Mouse dataset). The mice (C57BL/6, B&K Sollentuna, Sweden) were unilaterally lesioned with the neurotoxin 6-hydroxydopamine, and subsequently treated with saline or L-DOPA/benserazide according to previous protocol(22). All mice were killed by focused microwave irradiation using special equipment(22). Striatum (left and right) was dissected out for biochemical analyses and stored in -80°C until further analysis. The experiments were approved by the local ethical committee at Karolinska Institute (N282/06) and followed the European Communities Council Directive of November 24th 1986 (86/609/EEC).

Animal experiments (Rat dataset). Male Sprague-Dawley rats and male Wistar rats (B&K Sollentuna) were randomly divided into two groups of seven rats, prior to drug or vehicle injections. After 14 days when the morphine-treated rats were completely tolerant to the opiate they were challenged with a single dose of naloxone. The experiment ended 3.5 hours after the naloxone challenge by decapitation of the rats. At the end of the experiment, brains were removed and snap-frozen in -80°C cold isopentane and placed on dry ice to freeze thoroughly. The brain region of interest was the nucleus accumbens (core and shell). All animal procedures followed the guidelines of European Communities Council Directive (86/609/EEC) and were approved by the local ethical committee in Uppsala, Sweden.

Animal experiments (Quail dataset). Fertilized Japanese quail eggs were obtained from a local breeder. On day three of incubation, eggs were injected with 300 ng ethinylestradiol (EE_2) each. The EE_2 was dissolved in an emulsion of peanut oil, lecithin, and water. Controls received emulsion only. The holes in the shells were sealed with melted paraffin wax and the eggs were returned to the incubator. Diencephalon was excised as quickly as possible from embryonic brains on embryonic day (ed) 12 and ed17 and was placed in pre-weighed Eppendorf tubes. All samples were quickly frozen in liquid nitrogen, and stored at -80°C. The experiments where approved by the local Ethics Committee for Animal Research.

Tissue extraction and sample preparation.

The tissue samples were extracted by sonication in 0.25% acetic acid (Quail: 5 µl/mg tissue, Mouse, Rat: 7.5 µl/ mg tissue). In the Mouse dataset, the sonication step was performed in 95°C sample extraction buffer, followed by incubation in 95°C for one minute. In the Mouse and Rat experiment, the buffer was spiked with deuterated Met-enkephalin [YGG**F**M **F**(D8)], neurotensin Pyr-(Q)**L**YENKPRRPYI**L**, **L**(D3) and substance P [RPKPQQ**FF**GLM-amide **F**(D8)], at a concentration of 40 fmol/µl. Cell debris was spun down by centrifugation for 40 min at 14 000xg, 4°C and the supernatant was transferred to a filtering device (Microcon, YM-10) with a cut-off limit of 10 kDa. The peptide fraction was isolated through centrifugation for 60 min at 14 000xg, 4°C and stored at -80°C until further analysis.

Liquid chromatography. The peptide mixture was analyzed on a nano-LC system (Ettan MDLC, GE Healthcare, Uppsala) coupled to an electrospray Q-TOF (Waters, Manchester, UK) or a linear ion trap (LTQ, Thermo Electron, San José, USA) mass spectrometer. The sample (5µl) was injected and desalted on a pre-column (300 µm inner diameter (i.d.) x 5mm, C18 PepMapT, 5 µm, 100Å, LC Packings, Amsterdam, The Netherlands) at a flow rate of 10 µl/min for 10 min. A 15-cm fused silica emitter with a 75 µm i.d. and a 375 µm outer diameter (Proxeon Biosystems; Odense, Denmark) packed in-house with a slurry of reverse-phased Reprosil-Pur C18-AQ 3-µm resin (Dr. Maisch GmbH; Ammerbuch-Entringen, Germany) was used as the analytical column. The mobile phases used were Buffer A (0.25% HAc in water) and Buffer B (84% acetonitrile (ACN) and 0.25% HAc in water). The peptide samples were separated and eluted during a 40 min gradient from 3-60% (3-80% Quail) Buffer B at a flow rate of approximately 170 nl/min. Prior to each block a blank analysis (0.25% acetic acid) was carried out.

MS data acquisition. MS data was collected in-line with the nano-LC system. On the Q-TOF instrument, MS data was collected during 50 min in the m/z range 300-1000. On the LTQ, data was collected during 50 min and the acquisition was set up to continuously switch between full MS scan (m/z 300-2000), zoom scan (most intense peak in full scan) and MS/MS scan (most intense peak in zoom scan) in a data dependant manner.