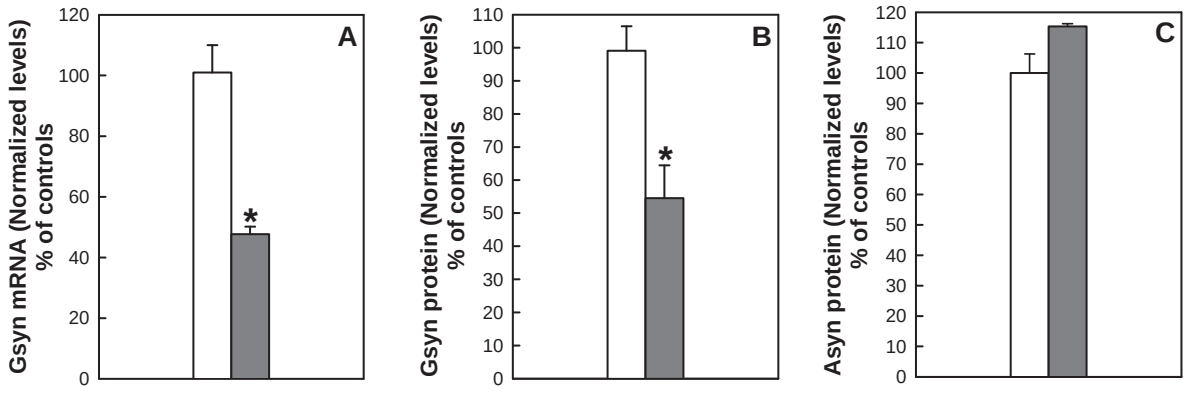

Supplementary figure 1

Supplementary Methods

Animals, virus injection and intravenous surgery

Male Sprague-Dawley rats (Charles River, St Germain/L'Arbresle, France), 280-300g at the beginning of the experiments, were single-housed at standard temperature (21°C) and in a light-controlled environment (12hr light/12hr dark, lights off at 7:00 AM) with *ad libitum* access to food and water. Under ketamine(10 mg/kg)/xylazine(0.1 mg/kg) anesthesia, rats were implanted with an intracardiac catheter into the jugular vein and bilaterally injected into the nucleus accumbens [anterior +1.4, lateral +/-1.2, ventral -6.8 from bregma and the dura mera[1]] with 2 µl of concentrated lentiviral stock LV-GFP (control, n=10) or LV-GSyn-siRNAs (n=10). Animals were administered gentamycin for four days following surgery (1 mg/ kg). The catheters were flushed with a saline–heparin mixture (100IU/ml) after each self-administration session. Experiments were performed in accordance with French (Ministère de l'agriculture et de la forêt, 87-848) and European Economic Community (EEC, 86-6091) guidelines for care of laboratory animals.

Self-administration set-up and procedures[2]

Nose-pokes into active (leading to cocaine delivery) and inactive (without scheduled consequences) holes were used as operant responses to evaluate cocaine SA. Cocaine infusions (cocaine HCL, Coopération Pharmaceutique Française, Bordeaux, France, dissolved in 0.9% NaCl) were administered at a rate of 20µl/sec over 2 sec. To prevent overdose, each cocaine infusion was followed by a 50sec time-out period during which active nose-pokes were without consequences. A cue light located above the active hole was activated 1 sec before each cocaine infusion for 4 sec.

Seven days after surgery, rats were trained for cocaine SA (0.8 mg/kg/infusion) during one daily 1-hr session. During this period, response requirement or fixed ratio (FR: number of active nose-pokes leading to one cocaine infusion) was progressively increased from 1 to 5 [1 day at fixed ratio 1 (FR1), 6 days at FR3, 5 days at FR5].

After behavior was acquired and stabilized (12 days), i.e. significant discrimination between active and inactive holes and stable number of self-infusions over at least three consecutive SA sessions, a dose-response function was performed. Four doses were successively tested in a descending order (0.4, 0.2, 0.1, and 0.05 mg/kg/infusion). Each dose was tested during at least 4 consecutive days and until the criterion for stabilization was met (stable number of self-infusions over at least three consecutive SA sessions). Then, after two days back at the training dose (0.8 mg/kg/infusion), a progressive ratio (PR) schedule was

applied (2 days at 0.8 mg/kg/infusion followed by 2 days at 0.4 mg/kg/infusion). PR SA sessions lasted for 3 hr or ended 1hr after the last infusion earned. Within PR sessions, the ratio requirement was progressively increased from 5 to 1985. The breakpoint corresponded to the last ratio completed and testified of the rat's motivation for cocaine.

Then cocaine- and cue-induced incentive effects were tested using a reinstatement procedure. Both tests were preceded by two sessions in training conditions. Cocaine-induced reinstatement was tested over two consecutive sessions. On day 1, following a 90-min period of extinction during which nose-pokes were without scheduled consequences, intravenous infusions of four different unit volumes (20, 40, 80 and 160 µl in ascending order) of a cocaine solution (0.2 mg/kg per 20µl) were successively triggered by the computer, one infusion every 30 min. Consequently, four doses of cocaine (0.2, 0.4, 0.8 and 1.6 mg/kg/infusion) corresponding to the four unit volumes were tested. On day 2, the same schedule was applied except that the cocaine solution was replaced by a vehicle solution. For each hole, responses were cumulated over the 30 min following each vehicle or cocaine infusion.

Cue-induced reinstatement was performed over one session and was induced by contingent exposures to the cocaine-associated cue. Rats were placed for 150 min in the SA chambers. During the first 90 min, an extinction procedure was applied during which nose-pokes were without scheduled consequences. During the following 60 min, the white cue light was contingently delivered for 4 sec and according to an FR5 in response to nose-pokes in the hole previously associated with both cocaine and the cue. The beginning of this procedure was signaled by one 4 sec non-contingent presentation of the cue light. For each hole, responses were cumulated over the 60 min of cue presentation.

Brain sampling and storage

Rats were decapitated. Brains were quickly removed from the skull. They were immediately sliced in coronal sections using a brain matrix (Harvard Apparatus, Holliston, MA) to dissect nucleus accumbens that were then rapidly frozen in liquid nitrogen and stored at –80°C until RNA (for qPCR) or protein (for western blots) extraction.

Quantification of GSyn and Asyn proteins by Western blots on nucleus accumbens samples (150–200 mg) were performed using a procedure previously described[3] using GSyn (1:1000; ab47966, Abcam, UK) or ASyn (1:1000, ab1904, Abcam, UK) as primary antibodies and HRP-conjugated goat anti-mouse IgG (1:2000, ab1188, Abcam, UK) as secondary antibody. Proteins were visualized using enhanced chemiluminescence (Millipore, Basel, Switzerland). Signals were detected with a Fluorchem tm 8900 apparatus (AlphInnotech, Witec,

Switzerland) and the TIFF pictures were quantified with the Multianalyst Software (Biorad, Switzerland). Membranes were washed for 30min in TBST (10 mM Tris, 150 mM NaCl, 0.1% Tween-20), then placed in stripping solution (Glycine-HCl 25 mM, pH 2.0, 1%SDS) for 30 min and treated as above for β-actin detection (1:4000; ab11801 Abcam, UK) for normalization.

***Statistical analyses*:** Results are expressed as mean ± SEM and were analyzed using Statistica 6.0 (Statsoft, OK, USA). One-way or repeated measures analysis of variance was used to determine possible group effects and interactions, with group (LV-GFP vs LV-GSyn-siRNAs) as between-subject factor and time (sessions or time blocks), FR, hole (active vs inactive) or doses (cocaine doses or vehicle volumes) as within-subject factors. Significant main effects or interactions were explored by a pairwise comparison of means using the Newman-Keuls *post hoc* test. For qPCR and western blot analyses, the two-tailed Student's t-test was applied to compare LV-GFP and LV-GSyn-siRNAs groups.

Supplementary references

1. G. Paxinos, C. Watson, *The Rat Brain in Stereotaxic Coordinates*. (New York Academic Press, 1998).
2. D. Belin, E. Balado, P. V. Piazza, V. Deroche-Gamonet, *Biol Psychiatry* **65**, 863 (May 15, 2009).
3. F. Boyer, J. L. Dreyer, *Eur J Neurosci* **26**, 2764 (Nov, 2007).

Supplementary results

Vehicle-induced reinstatement. We compared LV-GFP and LV-GSyn-siRNAs rats for their behavior during the extinction procedure preceding vehicle infusions. As during the first extinction procedure (before cocaine infusions), the two groups did not differ for discrimination, total and time-course responding.

Cue-induced reinstatement. Similarly, we compared LV-GFP and LV-GSyn-siRNAs rats for their behavior during the extinction procedure preceding cue presentations. As during the first two extinction procedures (before cocaine and vehicle infusions), the two groups did not differ for discrimination, total and time-course responding.

Furthermore, in addition to hole discrimination during the 60 minutes of cue presentation, reinstatement of self-administration by the cue was also analyzed by comparing responding during the last 60 minutes of extinction to responding during the 60 minutes of cue presentation. As expected, the cue produced a significant increase in responding [Cue effect, $F(1,15)=7.76$, $p<0.01$] which specifically concerned the active hole [Cue x Hole, $F(1,15)=13.99$, $p<0.001$]. This cue effect was similar in LV-GFP and LV-GSyn-siRNAs rats [Group effect, $F(1.15)=0.003$, $p=0.95$].

Legend to the Supplementary figure

Supplementary Figure 1: gamma- and alpha-synuclein expression in the nucleus accumbens of rats administered with LV-GSyn-siRNAs (grey bars) and rats administered with LV-GFP (controls, white bars). A. GSyn mRNA levels. Total accumbens RNA was extracted, quantified and tested for integrity, qPCR were performed and analyzed using the ΔCt method with β-actin as reference housekeeping gene. Comparisons were made between control and LV-GSyn-siRNAs rats using the ΔΔCt method. Ratio of expression between LV-GSyn-siRNAs and LV-GFP rats was calculated using the 2ΔΔCt method. For clarity, data were as percentage of LV-GFP rats (controls). Rats injected with LV-GSyn-siRNAs displayed a 52% of decrease in Gsyn mRNA levels as compared to controls ($p<0.001$). **B-C. Gsyn and ASyn protein levels.** Western blots on nucleus accumbens samples were performed using Gsyn or Asyn as primary antibodies and HRP-conjugated goat anti-mouse IgG as secondary antibody. As compared to LV-GFP control rats, rats injected with LV-GSyn-siRNAs in the nucleus accumbens displayed a 45.5% decrease in GSyn protein levels in this structure ($p<0.05$), while ASyn protein levels were not altered.