Supplemental Information

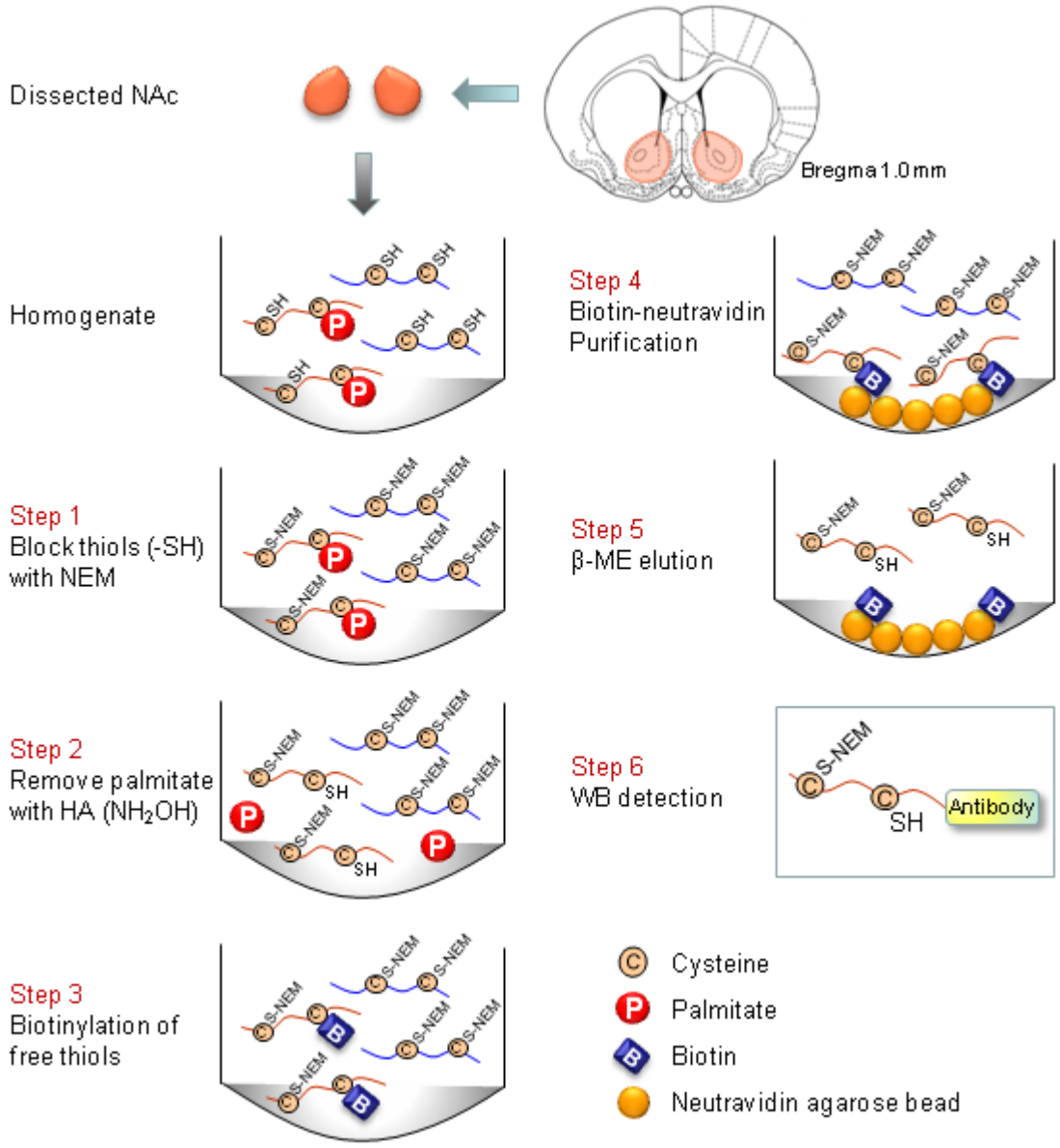

Figure S1. Stepwise ABE assays of protein palmitoylation. The assay is based on the principle that 1) removing palmitate from proteins gives rise to free sulfhydryls (-SH groups, thiols) at the specific palmitoylation sites, and 2) these proteins are then biotinylated at the newly–freed thiol sites and affinity purified for Western blot detection. In NAc homogenates, there are preexisting free cysteine thiols (-SH groups) which are blocked with NEM in sample preparations. HA is then added to cleave the palmitoylation thioester bond, but not the covalent attachment of NEM to cysteine because the latter is not sensitive to HA. The HA-produced free thiols are linked by the thiol-reactive biotin. Biotinylated proteins are affinity purified with neutravidin-agarose, followed by elution with β-ME. Eluted proteins are then individually identified in Western blot analysis with respective antibodies.

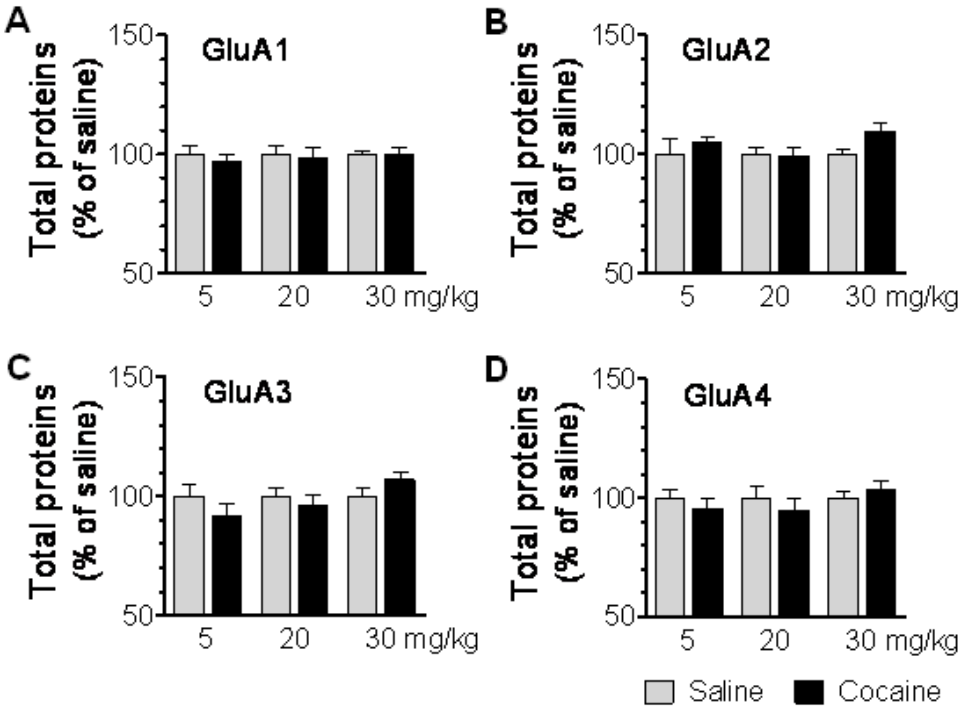

Figure S2. Effects of cocaine on total AMPAR subunits GluA1-4 in the rat NAc. Rats were given a single dose of cocaine or saline. They were sacrificed 30 min after drug injection for immunoblot analysis of total GluA1 (A), GluA2 (B), GluA3 (C), and GluA4 (D) expression. Data are presented as means ± SEM (n = 4-8 per group).

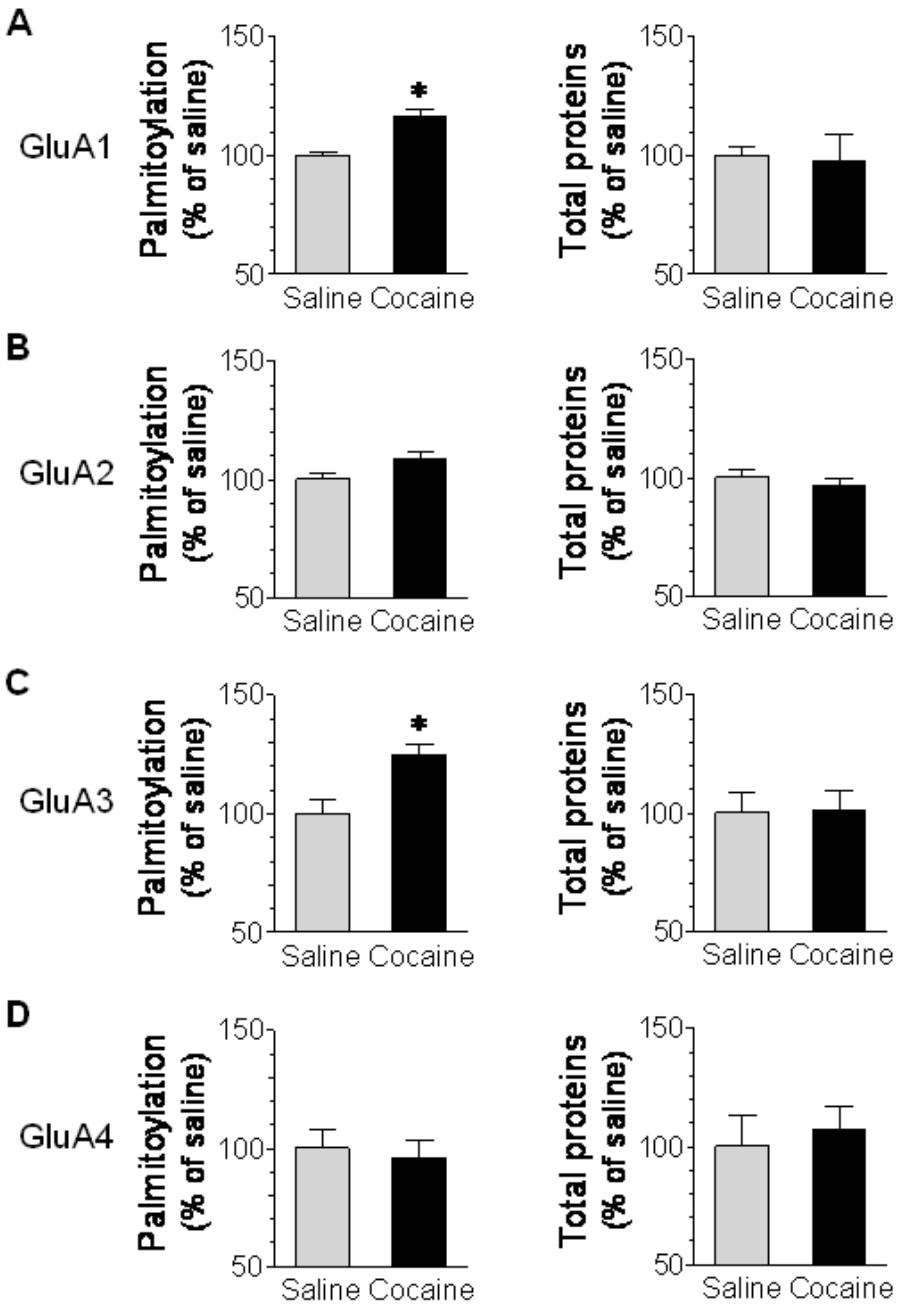

Figure S3. Effects of cocaine on palmitoylation of AMPAR subunits GluA1-4 in the rat dorsal striatum. Rats were given a single dose of cocaine (20 mg/kg, i.p.) or saline. They were sacrificed 30 min after drug injection for the ABE analysis of palmitoylation of GluA1 (A), GluA2 (B), GluA3 (C), and GluA4 (D). Data are presented as means ± SEM (n = 6-8 per group). **P* < 0.05 versus saline (Student *t* test).

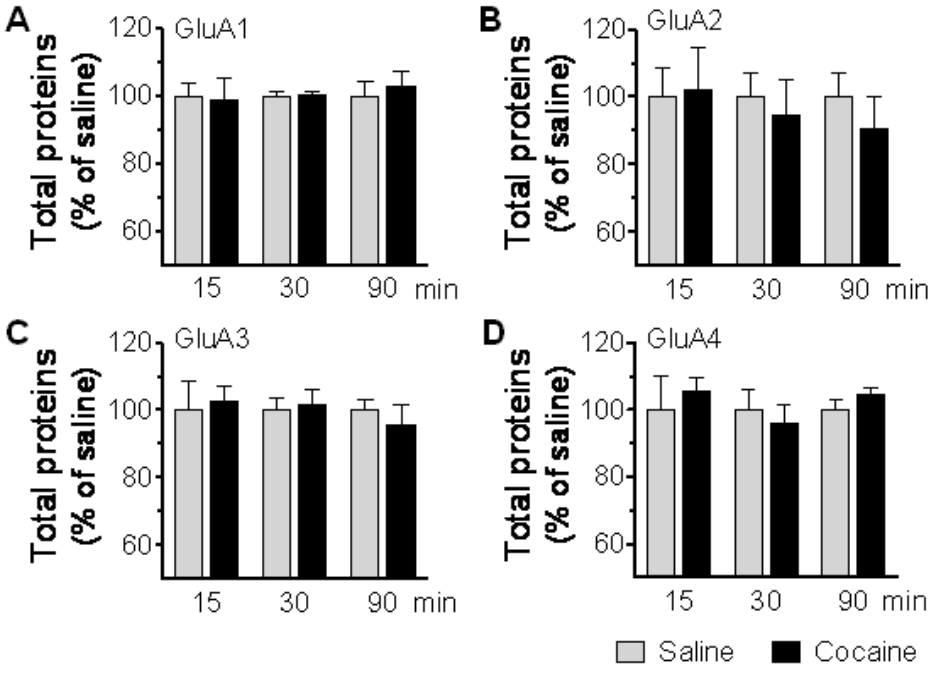

Figure S4. Effects of cocaine on total AMPAR subunits GluA1-4 in the rat NAc in a time-course study. Rats were given a single dose of cocaine (20 mg/kg, i.p.) or saline. They were sacrificed at different time points after drug injection for immunoblot analysis of total GluA1 (A), GluA2 (B), GluA3 (C), and GluA4 (D) expression. Data are presented as means ± SEM (n = 5-7 per group).

Supplemental Methods & Materials

Western blot. Western blot was carried out as described previously (1, 2). Briefly, equally-loaded proteins were separated on SDS NuPAGE Novex 4-12% gels (Invitrogen, Carlsbad, CA). Proteins were transferred to the polyvinylidene fluoride membrane (Millipore, Bedford, MA) and blocked in blocking buffer (5% nonfat dry milk in phosphate-buffered saline and 0.1% Tween 20) for 1 h. The blots were washed and incubated in the blocking buffer containing a primary rabbit antibody against GluA1 (Millipore, AB1504), GluA4 (Millipore, AB1508), GluN1 (Millipore, 06-311), or H-Ras (Santa Cruz, Santa Cruz, CA), or a mouse antibody against GluA2 (Millipore, MAB397), GluA3 (Millipore, MAB5416), or α-actinin (Millipore) usually at 1:200-2000 overnight at 4°C. This was followed by 1 h incubation in a horseradish peroxidase-linked secondary antibody against rabbit or mouse (Jackson Immunoresearch Laboratory, West Grove, PA) at 1:5000. Immunoblots were developed with ECL (Amersham Pharmacia Biotech, Piscataway, NJ), and captured into Kodak Image Station 2000R. Kaleidoscope-prestained standards (Bio-Rad, Hercules, CA) and MagicMark XP Western protein standards (Invitrogen) were used for protein size determination. The density of immunoblots was measured using the Kodak 1D Image Analysis software.

Primary striatal neuronal cultures and analysis of palmitoylation by metabolic labeling. GABAergic medium spiny neurons from the striatum of rat embryos (18 d) were prepared according to standardized procedures in this laboratory (3; 4). Cells were cultured for 14-18 d before use. For analysis of palmitoylation, a metabolic labeling method (5, 6) was used. Neurons were metabolically labeled with [^{3}H]palmitate (1 mCi/ml) for 24 h. They were then lysed in RIPA buffer and immunoprecipitated with an anti-GluA1 antibody (Millipore, AB1504). A small fraction of immunoprecipitated GluA1 (10%) was used for Western blot with the GluA1 antibody to confirm the immunoprecipitation results. A large portion (90%) was used for fluorography with film exposure at −80ºC. In the experiment with HA, duplicate gels of radiolabeled GluA1 were treated with 1 M HA (pH 7.0) or control 1 M Tris-HCl (pH 7.0) for 18 h at room temperature, followed by the film exposure.

Surgeries for intra-NAc injection. As described previously (2), rats were anesthetized with 2.5–3% isofluorane and placed in a stereotaxic holder. Two 26-gauge guide cannulae were

bilaterally implanted into the NAc (1–1.6 mm anterior to bregma, 1–1.6 mm lateral to midline, and 4.2–4.8 mm below surface of skull) based on the atlas of Paxinos and Watson (7). Rats were allowed 5–7 days for recovery. On the day of the experiment, a 33-gauge injector replaced the inner sealing wire and protruded 2.5 mm beyond the guide cannula. 2-bromopalmitate (2-BP) (Aldrich Chemical Co., St. Louis, MO) or vehicle dimethyl sulfoxide (DMSO) was infused into the NAc in freely moving rats in a volume of 0.5 μl. After completing injection, the cannula was left in place for 3 min. The standard histological examination was conducted after experiments to verify injection sites.

Assessments of cellular damage in striatal sections. Rats were anesthetized and sacrificed via an intracardial perfusion with 4% paraformaldehyde. Serial coronal sections (30–40 μm) were cut through the injection sites within the NAc. Assessments of cellular damage in sections were conducted using two techniques. First, pyknotic cells were counted in sections stained for cresyl violet. These cells were identified by dark staining, condensed chromatin, and pale or absent cytoplasm (8). Second, apoptotic nuclei were detected by using both morphological indicators and fragment end labeling via terminal deoxynucleotidyl transferase (FragEL kit, Calbiochem, San Diego, CA). A positive control for the two techniques was generated by treating rats with kainic acid (15 mg/kg, i.p.) and analyzing striatal sections at various time points.

Behavioral assessments. Behavioral activity was assessed with an infrared photo-cell-based, automated Opto-Varimex-Micro apparatus (Columbus Instruments, Columbus, OH) in a sound-attenuated room as described previously (1, 2). Motor activity was recorded at 5 min intervals before and after drug injection. Stereotypy was detected using computer-generated stereotypy time recorded by VersaMax monitors, which refers to the total time that stereotypy behaviors (repetitive breaks of a given beam or beams with an interval less than 1 s) were observed.

Statistics. The results are presented as means ± S.E.M. The data were evaluated using a one- or two-way ANOVA followed by a Bonferroni (Dunn) comparison of groups using least squares-adjusted means, or a Student *t* test, as appropriate. Probability levels of $P < 0.05$ were considered to be statistically significant.

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