Supplementary Figure Legends

Figure 1S. Subcellular fractionation analysis. Panel A shows the dissection of the medial prefrontal cortex (dorsal and ventral) and ventral tegmental area. The numbers on the coronal brain section represent distance from Bregma. Panel B shows a schematic of the subcellular fractionation procedure, as described in Material and Methods (adopted from Dunah and Standaert, 2001). The H and LP1 fractions were used for western blot analysis. Panel C shows representative immunoblots from nucleus accumbens tissue characterizing the content of each fraction. The membrane was probed with markers for presynaptic vesicles (synaptophysin), Golgi apparatus (GM 130), endoplasmic reticulum (calnexin), and postsynaptic density (PSD 95). The H fraction contains all of the probed subcellular compartment markers while the LP1 fraction is highly enriched in PSD 95, contains lesser amounts of synaptophysin, trace amounts of calnexin, and is devoid of GM 130.

Figure 2S. Cocaine and saline self-administration in Home, Box and Extinction rats used for protein analysis. Data represent the number of infusions acquired by rats subsequently assigned to the Home, Box, and Extinction post-SA conditions across the 14-day test period during which cocaine (Coc) or saline (Sal) infusions were available during daily 6-h self-administration sessions. No differences in saline or cocaine self-administration were observed between rats assigned to the various post-SA conditions.

Figure 3S. Effects of post-SA conditions on cocaine-seeking behavior. Data represent the number of infusions acquired during the 6-h sessions on the final day of self-administration

(SA), across a 9-day post-SA period and during a test session conducted after post-SA period in rats allowed to remain in the home cage with no further treatment (Home), rats exposed daily to the self-administration chambers in absence of exposed levers or other cocaine-associated cues (Box) and rats engaged in daily extinction training (Extinction). Cocaine-seeking behavior was significantly reduced in Extinction rats compared to rats exposed to Home or Box conditions (**$p<0.0001$ vs. Home and Box) and was significantly lower in rats exposed to Box conditions compared to rats exposed to Home conditions (*$p=0.026$).

Figure 4S. Tissue and postsynaptic density (synaptosomal membrane fraction) levels of Homer1b/c protein after cocaine self-administration and Home, Box, and Extinction treatments. See Figure 1 legend for details. The tissue and PSD levels of Homer1b/c protein were not changed following any post-SA treatment.

Figure 5S. Tissue and postsynaptic density (synaptosomal membrane fraction) levels of PICK1 protein after cocaine self-administration and Home, Box, and Extinction treatments. See Figure 1 legend for details. The tissue and PSD levels of PICK1 protein were significantly increased in Box group in vmPFC. Comparing cocaine self-administration groups across post-SA conditions, there were significant differences in tissue and PSD levels of PICK1 protein between Box and Home groups. *$p<0.05$ compared to respective saline control. #$p<0.05$ compared to cocaine Home group.

Figure 6S. Representative protein bands from dmPFC of saline and cocaine self-administration animals following post-SA treatments. See Methods for description of the experimental

conditions. The top panel shows results from the tissue protein analysis (H fraction). The bottom panel shows results from the synaptosomal membrane fraction (postsynaptic density) protein analysis (LP1 fraction).

Figure 7S. Representative protein bands from vmPFC of saline and cocaine self-administration animals following post-SA treatments. See Methods for description of the experimental conditions. The top panel shows results from the tissue protein analysis (H fraction). The bottom panel shows results from the synaptosomal membrane fraction (postsynaptic density) protein analysis (LP1 fraction).

Figure 8S. Representative protein bands from VTA of saline and cocaine self-administration animals following post-SA treatments. See Methods for description of the experimental conditions. The top panel shows results from the tissue protein analysis (H fraction). The bottom panel shows results from the synaptosomal membrane fraction (postsynaptic density) protein analysis (LP1 fraction).

Anatomical dissection and biochemical subcellular fractionation

At the end of the post-SA period, Home, Box, and Extinction rats were decapitated and brains quickly removed from the skull and placed in ice-cold saline for one minute. The tissue was blocked and coronal slices containing areas of interest were cut using a rat brain matrix (ASI, Warren, MI). The medial prefrontal cortex (mPFC) was blocked between plates 6 and 11 (Bregma 5.16-3.00 mm) (paxinos and Watson, 2005). The dorsal and ventral PFC border was set at the middle of prelimbic cortex based on previous anatomical and functional studies (Heidbreder and Groenewegen, 2003) and was hand dissected. The ventral tegmental area was blocked between plates 77-85 (Bregma -5.28-6.24 mm) and was hand dissected. All tissue samples were immediately frozen on dry ice and stored at -80 °C. The membrane preparation and biochemical fractionation were based on methods by Dunah and Standaert (2001) and Toda et al, (2003). A schematic of the membrane purification procedure, adopted from Dunah and Standaert (2001), is presented in Supplementary figure 1. The tissue was homogenized in ice-cold TEVP buffer containing (in mM) 10 Tris-HCl, pH 7.4, 5 NaF, 1 Na_3VO4, 1 EDTA, 1 EGTA, 320 sucrose, using a Dounce glass homogenizer. An aliquot of the whole tissue Dounce homogenate (H fraction) was collected, and the remainder was centrifuged at 1000 g to remove nuclei and large debris (P1). The supernatant (S1) was centrifuged at 10,000 g to obtain a crude synaptosomal fraction (P2) and subsequently was lysed hypo-osmotically for 30 min at 4 °C and centrifuged at 25,000 g to pellet a synaptosomal membrane fraction (LP1), which is enriched in postsynaptic density. All centrifugations were carried out at 4 °C. LP1 pellets were resuspended in TEVP. The H and LP1 fractions were solubilized with the addition of SDS to 1% (v/v) and

used for protein concentration measurement and Western Blot Analysis. All fractions were saved and stored at -80°C.

Western blot analysis

Two independent protein analyses were performed. In the first analysis, all saline-treated animals (n=10 per post-SA treatment group) were compared across post-SA conditions (i.e., 8 saline samples from Post-SA treatment conditions (2-3 samples from each Post-SA treatment) were loaded onto each gel and the mean percent changes compared to Home samples on the same gel were determined). This was done to identify any effects of post-SA conditions that were independent of cocaine exposure. The second analysis compared cocaine-treated animals (n=10) to their corresponding saline control group (n=10) for each post-SA treatment (i.e., 3-4 samples each from saline and cocaine treated animals were loaded onto each gel and the mean percent change resulting from cocaine self-administration was determined based on values for saline samples on the same gel). Due to the large number of samples, each of the three comparisons (10 saline and 10 cocaine for each post-SA treatment) was done separately.

Protein concentration was determined by the Lowry based DC protein assay (Bio-Rad, Hercules, CA). Protein samples were resolved by SDS-PAGE according to the method described by Ghasemzadeh et al., 2003. Briefly, proteins were transferred from the gel to a polyvinyldine fluoride (PVDF) membrane using a semi-dry transfer apparatus (Bio-Rad, Hercules, CA). Membranes were blocked for 2 hour at room temperature (Tris-buffered saline containing 3% non-fat dry milk) and incubated with commercially obtained primary antibody in antibody buffer (blocking buffer containing Tween-20, 50 μl/100 ml) overnight at 4 °C, washed with antibody buffer, and incubated with HRP-conjugated secondary antibody for 90 minutes at room

temperature. Membranes were washed and immunolabeling was visualized with enhanced chemiluminescence (ECL method). Chemiluminescent images were captured using a Kodak Image Station 4000MM. Band density was measured using Kodak Molecular Imaging Software v4.0. Commercially available antibodies were used to probe for the presence of proteins. The following primary antibodies were used in this study: mouse NMDAR1 (Millipore, Cat. # 05-432, 1:3000 dilution), rabbit GluR1 (Millipore, Cat. # 07-660, 1:10,000 dilution), rabbit mGluR5 (Millipore, Cat. # 06-451, 1:10,000 dilution), mouse PSD95 (Antibodies Inc., Cat. # 75-028, 1:10,000 dilution), mouse PICK1 (Antibodies Inc., Cat. # 75-040, 1:3000 dilution), rabbit Homer1b/c (Santa Cruz Biotech, Cat. # sc-20807, 1:3000 dilution), goat Actin (Santa Cruz Biotech, Cat. # sc-1615, 1:10,000). The following HRP-conjugated secondary antibodies were used: goat anti-rabbit IgG (Millipore, Cat. # 12-348, 1:20,000 dilution), goat anti-mouse IgG (Millipore, Cat. # 12-349, 1:20,000 dilution).

Statistics

The self-administration data (infusions/session) were evaluated using two-way ANOVA with repeated measures over sessions. Responding on test day (infusions/session) was compared using one-way ANOVA comparing the three post-SA treatment groups. In both cases, differences between groups were determined using *post hoc* Fisher's PLSD. For analysis of cocaine self-administration induced alterations in protein levels under each of the three post-SA conditions, immunoreactivity of protein samples from cocaine and saline rats was normalized as percent change relative to the respective mean saline control values for samples on the same gel. Normalized saline and cocaine values were then compared separately for each post-SA condition using Student’s t-tests. To permit comparison of cocaine-induced changes across post-SA

treatment conditions, saline self-administration effects on protein levels determined from a separate protein analysis were initially compared across post-SA conditions using one-way ANOVA followed by the Fisher's PLSD test to identify any effects of post-SA conditions that were independent of cocaine exposure. For this analysis saline self-administration induced protein alterations under each of the post-SA conditions were normalized as percent change relative to the respective mean Home sample saline values determined from the same gel. In cases where there were no differences following saline exposure across post-SA conditions, percent changes in protein following cocaine self-administration were compared across post-SA conditions using one-way ANOVA, followed by *post-hoc* testing using the Fisher's PLSD test. Statistical significance was set at $p < 0.05$.