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## Subcellular and subsynaptic localization of group I metabotropic glutamate receptors in the nucleus accumbens of cocaine-treated rats

D.A. Mitrano<sup>1,2</sup>, C. Arnold<sup>1</sup>, and Y. Smith<sup>1,3</sup>

<sup>1</sup> Yerkes National Primate Research Center, Emory University, Atlanta, GA 30322

<sup>2</sup> Molecular & Systems Pharmacology Graduate Program, Emory University, Atlanta, GA 30322

<sup>3</sup> Department of Neurology, Emory University, Atlanta, GA 30322

### Abstract

There is significant pharmacological and behavioral evidence that group I metabotropic glutamate receptors (mGluR1a and mGluR5) in the nucleus accumbens play an important role in the neurochemical and pathophysiological mechanisms that underlie addiction to psychostimulants. To further address this issue, we undertook a detailed ultrastructural analysis to characterize changes in the subcellular and subsynaptic localization of mGluR1a and mGluR5 in the core and shell of nucleus accumbens following acute or chronic cocaine administration in rats. After a single cocaine injection (30mg/kg) and 45 minutes withdrawal, there was a significant decrease in the proportion of plasma membrane-bound mGluR1a in accumbens shell dendrites. Similarly, the proportion of plasma membrane-bound mGluR1a was decreased in large dendrites of accumbens core neurons following chronic cocaine exposure (i.e. 1 week treatment followed by three weeks withdrawal). However, neither acute nor chronic cocaine treatments induced significant change in the localization of mGluR5 in accumbens core and shell, which is in contrast with the significant reduction of plasma membrane-bound mGluR1a and mGluR5 induced by local intra-accumbens administration of the group I mGluR agonist, DHPG. In conclusion, these findings demonstrate that cocaine-induced glutamate imbalance (Smith et al., 1995; Pierce et al., 1996; Reid et al., 1997) has modest effects on the trafficking of group I mGluRs in the nucleus accumbens. These results provide valuable information on the neuroadaptive mechanisms of accumbens group I mGluRs in response to cocaine administration.

### Keywords

immunogold; mGluR1a; mGluR5; nucleus accumbens; cocaine; DHPG

### INTRODUCTION

Changes in glutamate neurotransmission in the nucleus accumbens is a key neuroadaptive mechanism in response to acute or chronic cocaine exposure (Robinson and Berridge, 2003).

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Corresponding Author: Dr. Yolanda Smith, Yerkes National Primate Research Center, Emory University, 954 Gatewood Rd. NE, Atlanta, GA 30329, USA, E-mail: ysmit01@emory.edu.  
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*In vivo* microdialysis experiments have shown a significant increase in extracellular glutamate levels that peaks approximately 40 minutes following acute systemic cocaine injections in rats (Smith et al., 1995; Reid et al., 1997). In contrast, one week of chronic cocaine exposure, which leads to behavioral sensitization, followed by three weeks withdrawal, reduces basal extracellular glutamate levels by half compared to saline-treated animals (Pierce et al., 1996; Baker et al., 2003). These cocaine-induced effects on extracellular glutamate release lead to rapid  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor subunit internalization after acute cocaine and in contrast, increased AMPA receptor surface expression following chronic cocaine-induced behavioral sensitization (Boudreau and Wolf, 2005; Boudreau et al., 2007).

Metabotropic glutamate receptors (mGluRs) are divided into three classes based on pharmacological and structural properties. Group I mGluRs (mGluR1 and 5) are coupled to Gq and activate phospholipase C, increasing intracellular calcium and activating protein kinase C, while Group II (mGluR2 and 3) and Group III (mGluRs 4, 6, 7, and 8) mGluRs are coupled to Gi and inhibit cAMP formation (for review see (Conn and Pin, 1997)). Both group I mGluRs are widely distributed and partly co-localized in the nucleus accumbens (Mitrano and Smith, 2007) where they likely mediate some of the neuroadaptive changes associated with repeated cocaine administration. First and foremost is the fact that mGluR5 knockout mice do not self-administer cocaine and have decreased locomotor activity in response to cocaine administration despite a significant increase in dopamine release in the nucleus accumbens (Chiamulera et al., 2001). In line with these observations, systemic administration of mGluR5 antagonist reduces cocaine self administration in both rats and monkeys and attenuates the rewarding effects of cocaine in mice (McGeehan and Olive, 2003; Kenny et al., 2005; Lee et al., 2005). On the other hand, pretreatment with mGluR1 antagonist reduces behavioral sensitization to chronic cocaine administration in rats (Dravolina et al., 2006).

At the cellular level, modest, but significant and opposite, changes in mGluR5 protein and mRNA expression have been reported in the rat accumbens after chronic cocaine exposure and three weeks withdrawal (Ghasemzadeh et al., 1999; Swanson et al., 2001). A single *in vivo* exposure to cocaine abolishes endocannabinoid mGluR5-mediated retrograde long-term depression (LTD) and decreases the surface expression of mGluR5 in the mouse accumbens (Fourgeaud et al., 2004).

Most G-protein coupled receptors (GPCRs), including group I mGluRs, have the ability to travel to and from the plasma membrane in response to changes in extracellular levels of receptor agonists (see Gainetdinov et al., 2004 for review). In cell cultures and mice brain slices, mGluR1a and mGluR5 undergo agonist-stimulated internalization and endocytosis (Dale et al., 2001; Mundell et al., 2001) which, in some cases, was correlated with decreased group I mGluR-mediated physiological effects (Fourgeaud et al., 2004). However, there has been no *in vivo* study looking at changes in the trafficking of group I mGluRs following glutamate or receptor agonist stimulation in the mammalian brain.

Therefore, to address this issue, we undertook an in-depth ultrastructural analysis of changes in the subcellular and subsynaptic localization of mGluR1a and mGluR5 in the core and shell of the nucleus accumbens in rats acutely or chronically treated with cocaine and in animals that received local intracerebral injection of group I mGluR agonist.

## EXPERIMENTAL PROCEDURES

### Animals and cocaine treatments

Thirty-three male Sprague Dawley rats weighing 225–250 grams upon arrival were used for the cocaine treatment experiments in this study. All procedures were approved by the animal

care and use committee of Emory University and conform to the U.S. National Institutes of Health guidelines. The chronic cocaine administration regimen used in this study was developed by Kalivas and colleagues (Kalivas et al., 1988) and is routinely used to induce behavioral sensitization to psychostimulants in rats. In brief, chronically treated rats were given either an i.p. injection of 0.9% saline or 15mg/kg cocaine on days 1 and 7 of the treatment period and locomotor activity was measured every 5 minutes for 2 hours by an IBM computer using Digipro software in photocell cages (Omnitech Electronics) equipped with 32 photobeams 5 cm above the floor. On days 2–6 of the treatment period, rats were given an i.p. injection of either 0.9% saline or 30mg/kg cocaine. Following the one-week treatment period, rats were left in their home cages for three weeks after the last injection, as a withdrawal period, and then sacrificed. Only rats that showed behavioral sensitization (i.e. animals that displayed a statistically significant increase in total locomotor activity on day 7 compared to day 1, data not shown) were used in the chronically cocaine-treated group.

Two groups of acutely treated rats were given a single i.p. injection of either 0.9% saline or 30mg/kg cocaine, put back in their home cages and then sacrificed either 45 minutes or 24 hours later. These time points were chosen based on previous studies that showed glutamate levels peak at approximately 40 minutes following acute cocaine (Smith et al., 1995; Reid et al., 1997) and changes in mGluR5 expression 24 hours following a cocaine exposure (Fourgeaud et al., 2004).

### Tissue preparation

For perfusion, all animals were deeply anesthetized with a cocktail of ketamine (60–100mg/kg, i.p.) and dormitor (0.1mg/kg, i.p.). The animals were then transcardially perfused with cold oxygenated Ringer's solution followed by a fixative containing 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer (0.1M; pH 7.4). Following perfusion, brains were removed from the skull, post-fixed in 4% paraformaldehyde for 24 hours, cut into 60- $\mu$ m-thick sections using a vibrating microtome and stored in phosphate-buffered saline (PBS; 0.01M, pH 7.4) at 4°C until further processing for immunocytochemistry. Prior to the immunocytochemical reactions, all sections were put into a 1% sodium borohydride solution for 20 minutes and then washed with PBS.

### Primary Antibodies

A commercially available monoclonal antibody against calbindin-D<sub>28k</sub> (Sigma, St. Louis, MO; Cat# C-9848, Lot# 082k4879) was used at a concentration of 1:5000 to distinguish between the accumbens shell and core. The calbindin-D<sub>28k</sub> antibody is derived from CB-955 hybridomas produced by fusion of mouse myeloma cells and splenocytes from BALB/c mice that were immunized with purified bovine kidney calbindin-D<sub>28k</sub>. The specificity of this antibody has been demonstrated through preadsorption immunohistochemical assays that abolish calbindin labeling (Celio, 1990), through Western blot analysis of rat brain tissue which shows a distinct band at 28kD (Miyata et al., 2000) and through immunohistochemistry which shows calbindin immunostaining in brain regions known to express a significant level of calbindin-D<sub>28k</sub> mRNA (Celio et al., 1990; Miyata et al., 2000; Winsky et al., 1989).

To localize mGluR1a, an affinity-purified rabbit polyclonal antibody against the C-terminus of rat mGluR1a (PNVTYASVILRDYKQSSSTL) conjugated to KLH with glutaraldehyde was used at a concentration of 1:1000 (Chemicon, Temecula, CA; Cat# AB1551, Lot# 21100471). In Western blot analysis by the manufacturer, this antibody labels a single band of ~140kD. Previous studies from our lab and others have used a combination of knockout mice, transfected HEK-293 cells, and preadsorption to determine the specificity of this mGluR1a antiserum. These studies showed that brain tissue from mGluR1a knockout mice did not display any specific mGluR1a labeling compared to wild-type. In addition, immunoblotting of cells

transfected with mGluR1a, but not mGluR5, labeled a band of 140kD (Kuwajima et al., 2004). Preadsorption studies in rat retina cells abolished mGluR1a labeling (Koulen et al., 1997).

An affinity-purified synthetic rabbit polyclonal antibody against the C-terminus of mGluR5 with a lysine added to the N-terminus (KSSPKYDTLIIRDYTNSSSSL) in a concentration of 1:5000 (Upstate Biotechnology, Lake Placid, NY; Cat# 06-451, Lot# 27884) was used to label mGluR5. According to the manufacturer's immunoblot analysis, the mGluR5 antibody labels a band of ~130kD. Specificity of the mGluR5 antibody has been shown in previous studies from our laboratory using knockout mice, transfected cells and homogenates of rat brain. These studies showed that brain tissue from mGluR5 knockout mice do not stain for mGluR5 and HEK-293 cells transfected with mGluR5 label a band of the correct molecular weight (Kuwajima et al., 2004). Furthermore, immunoblot analysis on proteins isolated from various brain regions labels a band that corresponds to the size of mGluR5 in regions known to express mGluR5 protein and mRNA (Mannaioni et al., 2001).

### Immunoperoxidase labeling for light microscopy

Following sodium borohydride treatment, sections were incubated for 1 hour at room temperature (RT) in PBS containing either 10% normal goat serum (NGS; for group I mGluRs), or normal horse serum (NHS; for calbindin-D<sub>28k</sub>), 1% BSA, and 0.3% Triton X-100, followed by the primary antibody solution containing 1% NGS or NHS, 1% BSA, and 0.3% Triton X-100 in PBS for 24 hours at RT. After three rinses in PBS, sections were incubated in secondary biotinylated goat anti-rabbit or horse anti-mouse IgGs at a concentration of 1:200 (Vector Laboratories, Burlingame, CA) for 90 minutes. The sections were rinsed again in PBS and then incubated another 90 minutes with the avidin-biotin peroxidase complex (ABC) at a dilution of 1:100 (Vector Laboratories). Finally, the sections were washed in PBS and TRIS buffer (50mM; pH 7.6) and transferred to a solution containing 0.025% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St Louis, MO), 10mM imidazole, and 0.005% hydrogen peroxide in TRIS buffer for 10 minutes. Sections were rinsed in PBS, mounted onto gelatin-coated slides, dehydrated and then coverslipped with Permount. Tissue was examined with a Leica DMRB microscope (Leica Microsystems, Inc., Bannockburn, IL) and images were taken using a CCD camera (Leica DC500) which was controlled by Leica IM50 software.

### Preembedding Immunoperoxidase labeling for electron microscopy

Following sodium borohydride treatment, sections were placed in a cryoprotectant solution for 20 minutes (PB 0.05M, pH 7.4, 25% sucrose, and 10% glycerol), frozen at -80°C for 20 minutes, returned to a decreasing gradient of cryoprotectant solutions, and rinsed in PBS. Sections were then incubated in primary and secondary antibody solutions, identical to those used for light microscopy; with two exceptions: 1) the omission of Triton X-100 and 2) incubation in primary antibody for 48 hours at 4°C.

After the DAB reaction, the tissue was rinsed in PB (0.1M, pH 7.4) and treated with 1% OsO<sub>4</sub> for 20 minutes. It was then returned to PB and dehydrated with increasing concentrations of ethanol. When exposed to 70% ETOH, 1% uranyl acetate was added to the solution for 35 minutes to increase the contrast of the tissue at the electron microscope. Following dehydration, sections were treated with propylene oxide and embedded in epoxy resin for 12 hours (Durcupan ACM, Fluka, Buchs, Switzerland), mounted onto slides and placed in a 60°C oven for 48 hours. Separate samples of the nucleus accumbens core and medial shell were cut out of the larger sections, mounted onto resin blocks and cut into 60-nm sections using an ultramicrotome (Leica Ultracut T2). The 60-nm sections were collected on Pioloform-coated copper grids, stained with lead citrate for 5 minutes to enhance tissue contrast and examined on the Zeiss EM-10C electron microscope. Electron micrographs were taken and saved with

a CCD camera (DualView 300W; Gatan, Inc., Pleasanton, CA) controlled by DigitalMicrograph software (version 3.10.1, Gatan, Inc.). Some of the digitally acquired electron micrographs were adjusted only for brightness or contrast using either the DigitalMicrograph software or Adobe Photoshop software (version 8.0, Adobe Systems Inc.). Micrographs were then compiled into figures using Adobe Illustrator (version 11.0, Adobe Systems Inc.).

### Preembedding Immunogold labeling for electron microscopy

Following sodium borohydride and cryoprotectant treatments, sections were incubated for 30 minutes in PBS containing 5% dry milk at RT and then rinsed in TBS-gelatin buffer (0.02M and pH 7.6). Sections were then transferred to primary antibody solutions with 1% dry milk in TBS-gelatin buffer for 24 hours at room temperature and then rinsed again in TBS-gelatin. After rinses, sections were treated for 2 hours at RT with secondary goat anti-rabbit IgGs conjugated with 1.4nm gold particles at a concentration of 1:100 (Nanoprobes, Yaphank, NY) diluted with 1% dry milk in TBS-gelatin. Sections were rinsed in TBS-gelatin and 2% sodium acetate buffer before gold particles were silver intensified to 30–50nm using the HQ silver kit (Nanoprobes) for approximately 10 minutes. The sections were then treated according to the same protocol of osmification, dehydration, embedding, and tissue selection described above for the immunoperoxidase procedure including the following changes: 1) the tissue was kept in 0.5% OsO<sub>4</sub> for 10 minutes instead of 20 and 2) the tissue was stained with 1% uranyl acetate for 10 minutes instead of 35.

### DHPG Injections

In order to ensure that the immunogold method used in our study was sensitive enough to detect changes in plasma membrane-bound group I mGluRs immunoreactivity in response to direct receptor agonist exposure in vivo, we examined changes in the subcellular localization of mGluR1a and mGluR5 in the accumbens of rats following local administration of group I mGluRs agonist. Six male Sprague-Dawley rats were anesthetized with isoflurane and fixed in a stereotaxic frame (Kopf; Tugunga, CA). Using a 10 $\mu$ l Hamilton syringe (Hamilton; Reno, NV), 2 $\mu$ l of either 1 $\mu$ mol (*RS*)-3,5-dihydroxyphenylglycine (DHPG; Tocris, Ballwin, MO) dissolved in 0.9% saline or 0.9% saline was injected over a 5 minute period into the nucleus accumbens core and medial shell based on coordinates from Paxinos and Watson (1998; +1.7mm AP, +1.3mm ML, -7.4mm DV). Rats were sacrificed 45 minutes after injections. This time point was chosen based on previous internalization studies of other GPCRs in response to agonist stimulation (Bernard et al., 1998; Dumartin et al., 1998; Bernard et al., 1999; Csaba et al., 2001). To assess the accuracy of the DHPG injection, the striatal tissue from these animals was serially cut, and 1 out of 4 sections were Nissl-stained to reveal the exact placement of the syringe. Tissue from animals in which the injection site was in the core and/or medial shell of the accumbens, was prepared for single preembedding immunogold labeling as described above.

### Analysis of Material

**Immunoperoxidase labeling for group I mGluRs**—Data for single immunoperoxidase labeling were collected from a total of 132 blocks of tissue, 1 block/animal in the medial shell (referred to as shell in the following sections) and 1 block/animal in core immunostained for either mGluR1a and mGluR5 from 7 rats chronically treated with cocaine, 7 rats chronically treated with saline, 10 rats acutely treated with cocaine, and 10 rats acutely treated with saline. Serial ultrathin sections taken from each of the blocks were examined and 35–40 electron micrographs of randomly selected immunoreactive elements were digitized at 25,000X. This resulted in a total surface of 18,027 $\mu$ m<sup>2</sup> of accumbens tissue to be examined for mGluR1a in saline-treated rats and 15,700 $\mu$ m<sup>2</sup> in cocaine-treated rats; 14,305 $\mu$ m<sup>2</sup> for mGluR5 in saline-

treated rats and  $15,235\mu\text{m}^2$  in cocaine-treated rats. Labeled elements were categorized as dendrites, spines, unmyelinated axons, myelinated axons, and axon terminals on the basis of ultrastructural features described by Peters et al. (1991). The density of labeled elements for each receptor subtype was calculated in the shell and core by dividing the number of labeled elements by the area of tissue examined. The means  $\pm$  SEM of labeled elements for the different animal groups were calculated and presented as bar histograms. Significant differences were assessed using Sigma stat software (version 2.03, SPSS Inc.) for one-way ANOVAs and Tukey's post-hoc test. The density of labeled elements was compared across each neuronal element between the cocaine- and saline-treated animals within each receptor subtype.

**Preembedding immunogold labeling for group I mGluRs**—Immunogold data were collected from 125 blocks of mGluR1a- and mGluR5-immunostained shell and core tissue as described above. Serial ultrathin sections from the surface of the blocks were collected and 35–40 electron micrographs of randomly selected immunoreactive elements were taken at 25,000X, for a total tissue surface area of  $13,316\mu\text{m}^2$  of accumbens tissue to be examined for mGluR1a in saline-treated rats and  $13,840\mu\text{m}^2$  in cocaine-treated rats;  $12,502\mu\text{m}^2$  for mGluR5 in saline-treated rats and  $14,247\mu\text{m}^2$  in cocaine-treated rats. Labeled elements were classified as described above with the addition that dendrites were further categorized arbitrarily as large (cross-sectional diameter greater than  $0.75\mu\text{m}$ ) or small (cross-sectional diameter equal to or less than  $0.75\mu\text{m}$ ). Gold particles were classified as either intracellular or plasma membrane-bound depending on their localization relative to the plasma membrane. To be categorized as plasma membrane-bound, gold particles had to be in contact with the membrane; all other particles were considered intracellular. The total number (i.e. 100%) of gold particles for each animal is equal to the number of intracellular + plasma membrane-bound gold particles examined. The mean ( $\pm$ SEM) percent of plasma membrane-bound gold particles was then calculated across all animals and presented as a bar histogram; the remaining gold particles were found in the intracellular compartment. Data were analyzed for significant differences between saline- and cocaine-treated animals using Sigma Stat software by one-way ANOVAs and Tukey's post-hoc test. Plasma membrane-bound gold particles were further classified into three categories; perisynaptic (touching or within a 20 nm range of the edges of postsynaptic specializations); synaptic (in contact with the main body of postsynaptic specializations); or extrasynaptic (on the plasma membrane but not associated with synapses).

**Preembedding immunogold labeling for group I mGluRs following DHPG Injections**—Immunogold labeling data were collected from 24 blocks of mGluR1a- and mGluR5-immunostained shell and core tissue as described above. To make sure the tissue examined had been exposed to DHPG, all blocks were taken within a range of 0.2 to 0.5mm from the tip of the injection syringe and from sections no more than  $120\mu\text{m}$  away in the rostral/caudal plane. Serial ultrathin sections from the surface of the blocks were collected and 35 electron micrographs of randomly selected immunoreactive elements were taken at 25,000X, for a total tissue surface area of  $2,440\mu\text{m}^2$  of accumbens tissue to be examined for mGluR1a in saline-treated rats and  $2,440\mu\text{m}^2$  in DHPG-treated rats;  $2,440\mu\text{m}^2$  for mGluR5 in saline-treated rats and  $2,440\mu\text{m}^2$  in DHPG-treated rats. The quantitative analysis of gold labeling distribution was the same as described in the previous section.

## RESULTS

### Light Microscopic Observations

Tissue containing the nucleus accumbens was stained separately for calbindin  $D_{28k}$ , mGluR1a or mGluR5. As shown in our previous study (Mitrano and Smith, 2007) and others (Meredith et al., 1996), calbindin immunoreactivity clearly delineated the boundaries of the shell and core

of the nucleus accumbens, hence serving as a guide for the selection of blocks of tissue in subsequent electron microscopic experiments. Tissue from all three cocaine treatment groups and saline-treated animals were immunostained for the two group I mGluRs. In line with our previous study, the immunoreactivity for mGluR1a and mGluR5 was distributed heavily in the neuropil of the shell and core of the accumbens, with very light labeling in cell bodies (Mitrano and Smith, 2007). No obvious difference in the overall distribution of mGluR1a and mGluR5 immunoreactivity was found in the nucleus accumbens between cocaine- and saline-treated rats (data not shown).

## Electron Microscopic Observations

**Cellular and Subcellular Localization of mGluR1a and mGluR5 in Cocaine-treated Groups**—Because both mGluR1a and mGluR5 immunoreactivity is found in the neuropil of accumbens tissue, high resolution electron microscopy is essential to assess potential changes in the cellular, subcellular and subsynaptic localization of these receptors between normal and cocaine-treated rats. First, we looked at the subcellular localization of both group I mGluRs in the three groups of cocaine-treated rats and control saline-treated animals using immunoperoxidase labeling for mGluR1a or mGluR5. The majority of labeling for both receptor subtypes in each experimental group was found in dendrites and spines, and less frequently in unmyelinated axons. Electron micrographs of immunoreactive elements from each treatment group are shown in figure 1, while quantitative data for these observations are depicted in figure 2 as the average density of labeled elements per 100 square microns ( $\pm$ SEM) of accumbens shell or core tissue. Overall, there was no significant difference in the distribution of mGluR1a- or mGluR5-immunoreactive neuronal elements between the different cocaine treatment groups and controls, regardless of receptor type, time point, or region of the accumbens examined using a series of one-way ANOVAs (Fig. 2).

**Subsynaptic Localization of mGluR1a and mGluR5 in Cocaine-treated Groups**—Because the immunoperoxidase method results in an amorphous diffuse reaction product within labeled elements, this method is not suitable to determine the exact subsynaptic localization of group I mGluRs in accumbens neurons. We therefore used the preembedding immunogold method that allows for a higher level of spatial resolution to detect changes in the subsynaptic localization of mGluR1a and mGluR5 immunoreactivity in response to chronic and acute cocaine treatment regimens. Examples of immunogold-labeled elements analyzed for this part of the study are illustrated in figure 3. The quantitative data, which provide the percentage of plasma membrane-bound gold particles ( $\pm$ SEM) labeling for mGluR1a or mGluR5 on dendrites and spines for the different treatment groups are presented in figure 4. Because the majority of gold labeling for mGluR1a and mGluR5 was found postsynaptically in dendrites and spines, the quantitative analysis of immunoreactivity was focused on these elements.

In the 45 minute acute treatment group, a significant decrease in the percent of plasma membrane-bound mGluR1a in large ( $30.9\pm 3.0\%$  saline vs.  $21.3\pm 1.9\%$  cocaine,  $F(3,15)=3.3$ ,  $n=5$ ,  $p<0.05$ ) and small ( $47.6\pm 1.0\%$  saline vs.  $35.2\pm 2.6\%$  cocaine,  $F(3,15)=4.9$ ,  $n=5$ ,  $p<0.05$ ) dendrites in the accumbens shell was found in cocaine-treated rats compared to controls, but no noticeable change in mGluR5 localization was found in these animals (Figs 3A,B; 4 A,B). In the 24 hours post-treatment group, neither mGluR1a or mGluR5 displayed any significant change in their pattern of subsynaptic localization compared to controls (Figs 3C; 4 C, D). Finally, a significant decrease in the percent of plasma membrane-bound mGluR1a immunoreactivity was found in large dendrites in the accumbens core of chronically cocaine-treated rats ( $48.0\pm 1.8\%$ ,  $n=7$ ) compared to controls ( $56.7\pm 2.3\%$ ,  $F(3,21)=3.1$ ,  $n=6$ ;  $p<0.05$ ), but no significant change in the subsynaptic distribution of either group I mGluR subtypes was found in spines or small dendrites in these animals.

The pattern of subsynaptic labeling on the plasma membrane was further classified into extrasynaptic, perisynaptic and synaptic. As shown in tables 1 and 2, there was no significant difference in the pattern of subsynaptic labeling between all treatment groups. In each group, more than 80% plasma membrane labeling in spines and 96–98% labeling in dendrites was extrasynaptic. A slightly larger incidence of perisynaptic labeling to asymmetric synapses was seen in spines compared to dendrites.

**Agonist-induced changes in the subsynaptic localization of group I mGluRs in the nucleus accumbens**—

Despite the well-established increases or decreases in extracellular glutamate in the nucleus accumbens (Smith et al., 1995; Pierce et al., 1996; Reid et al., 1997; Baker et al., 2003), only modest changes in group I mGluRs distribution were induced following acute or chronic cocaine treatment (see above). A potential interpretation for these observations might be that the pre-embedding immunogold method is not sensitive enough to detect changes in the subcellular and subsynaptic localization of group I mGluRs induced by changes in extracellular glutamate in cocaine-treated rats. In order to validate our approach, we assessed changes in the distribution of the two group I mGluRs in the rat accumbens forty-five minutes after intra-accumbal injection of the group I mGluR agonist, DHPG (Fig. 5C). Consistent with previous studies that showed internalization of various GPCRs following agonist stimulation (Bernard et al., 1998; Dumartin et al., 1998; Bernard et al., 1999; Csaba et al., 2001), local DHPG application induced significant decreases in the percentage of plasma membrane-bound labeling for mGluR1a or mGluR5 in specific neural elements in the shell or core of the rat accumbens (Fig. 5). In the shell, a significant decrease in plasma membrane-bound mGluR1a immunoreactivity was found in small dendrites ( $61.0 \pm 4.1\%$  saline vs.  $40.3 \pm 2.8\%$  DHPG,  $F(3,8)=11.2$ ,  $n=3$ ,  $p<0.05$ ) and spines ( $83.3 \pm 1.6\%$  saline vs.  $40.9 \pm 2.4\%$  DHPG,  $F(3,8)=96.5$ ,  $n=3$ ,  $p<0.001$ ), while plasma membrane-bound labeling for mGluR5 was significantly reduced in all neural compartments examined (large dendrites:  $53.5 \pm 8.2\%$  saline vs.  $26.3 \pm 4.9\%$  DHPG,  $F(3,8)=7.0$ ,  $n=3$ ,  $p<0.05$ ; small dendrites:  $58.5 \pm 4.1\%$  saline vs.  $43.4 \pm 0.5\%$  DHPG,  $F(3,8)=12.0$ ,  $n=3$ ,  $p<0.05$ ; spines:  $77.2 \pm 3.4\%$  saline vs.  $54.9 \pm 3.8\%$  DHPG,  $F(3,8)=12.4$ ,  $n=3$ ,  $p<0.01$ ). The pattern was quite different in the accumbens core where a significant decrease in the percent of plasma membrane-bound mGluR1a was found in large dendrites ( $55.0 \pm 7.5\%$  saline vs.  $32.3 \pm 2.1\%$  DHPG,  $F(3,8)=5.8$ ,  $n=3$ ,  $p<0.05$ ) and spines ( $78.7 \pm 1.7\%$  saline vs.  $55.4 \pm 2.2\%$  DHPG,  $F(3,8)=96.5$ ,  $n=3$ ,  $p<0.001$ ), but not in small dendrites. As for mGluR5, no significant change in the percentage of plasma membrane-bound labeling was found in the core.

## DISCUSSION

Two main conclusions can be drawn from this study. First, in contrast to the strong response of ionotropic AMPA receptors (Boudreau and Wolf, 2005; Boudreau et al., 2007), group I mGluRs in the nucleus accumbens display modest changes in their subcellular and subsynaptic localization after chronic or acute cocaine administration. Second, both mGluR1a and mGluR5 show a significant degree of internalization following local extracellular application of the group I mGluR agonist DHPG in both the core and shell of the nucleus accumbens. Together, these observations suggest that the trafficking of AMPA and group I mGluRs is differently affected by acute or chronic cocaine-induced changes in extracellular glutamate in the nucleus accumbens (Smith et al., 1995; Pierce et al., 1996; Reid et al., 1997). Furthermore, in line with other GPCRs (Bernard et al., 1998; Dumartin et al., 1998; Bernard et al., 1999; Csaba et al., 2001), our data provide the first in vivo evidence, in the brain, that group I mGluRs in the nucleus accumbens are endowed with trafficking properties that allow for rapid internalization in response to strong agonist stimulation.

One explanation for the minimal effect of cocaine administration on group I mGluRs trafficking could be that the cocaine administration regimen used in our study did not induce changes in



extracellular glutamate in the nucleus accumbens. However, this is unlikely based on many previous studies showing rapid changes in glutamate release in the rat accumbens following administration regimens of acute or chronic cocaine similar to those used in the present study. For instance, extracellular glutamate levels in the accumbens are increased by as much as 300% over baseline approximately 40 minutes after a single injection of 30 mg/kg cocaine (Smith et al., 1995; Reid et al., 1997). In contrast, the behavioral sensitization paradigm of 7 days of chronic cocaine exposure followed by three weeks withdrawal results in almost 50% reduction in extracellular glutamate compared to controls (Pierce et al., 1996; Hotsenpiller et al., 2001; Baker et al., 2003). Although not fully understood, there is good evidence that increased synaptic release of neurotransmitter from prefrontal cortical afferents may be the main source of extracellular glutamate buildup following acute cocaine administration (McFarland et al., 2003), while behavioral sensitization to chronic cocaine exposure decreases the activity of the cystine/glutamate exchanger, thereby, lowering extracellular glutamate levels in sensitized rats (Baker et al., 2003). In light of these findings, we can, therefore, assume that the levels of glutamate in the nucleus accumbens of rats used in the present study have been altered in the same manner by acute and chronic cocaine exposure.

A significant 15–20% increase in mGluR5 mRNA, but 10% decrease in mGluR5 protein expression along with blunted functioning of mGluR1 in the medial accumbens was reported in chronically cocaine-treated rats (Ghasemzadeh et al., 1999, Swanson 2001). On the other hand, 24 hours following a single cocaine injection, endocannabinoid-mediated LTD, regulated by mGluR5, is abolished and accompanied by a 50% reduction in surface expression of mGluR5 in mice accumbal medium spiny neurons (Fourgeaud et al., 2004). Furthermore, Homer 1b/c, one of the scaffolding proteins that regulates the trafficking and plasma membrane expression of group I mGluRs in vitro (Roche et al., 1999; Ango et al., 2002), is decreased by about 20% in the accumbens of behaviorally sensitized rats three weeks following chronic cocaine exposure (Swanson et al., 2001).

Apart from minor changes of dendritic mGluR1a distribution in the 45 minutes acutely cocaine-treated group and the chronically cocaine-treated animals, our electron microscopic study did not reveal any significant change in the overall pattern of subcellular and subsynaptic localization of mGluR1a or mGluR5 in response to either regimens of cocaine administration. Because the regulation of extracellular glutamate that reaches extrasynaptic group I mGluRs may be very complex and rely on many factors including the amount of glutamate released from axon terminals, amount of glutamate released by astrocytes and amount of glutamate reuptake by glial and neuronal glutamate transporters (Brasnjo and Otis, 2001; Oliet et al., 2001; Reichelt and Knopfel, 2002; Rusakov and Lehre, 2002), it is hard to predict how much glutamate, indeed, reaches these mGluRs. As shown in the cerebellum, there could be very subtle subsynaptic regulation of glutamate by neuronal transporters that strongly modulate the activity of group I mGluRs (Brasnjo and Otis, 2001). If such regulatory processes are in place in the nucleus accumbens, changes in extracellular glutamate levels measured with microdialysis do not provide an accurate index of the exact concentration of transmitter individual group I mGluRs may be exposed to following cocaine administration.

Our results are different from those of Fourgeaud et al. (2004) showing a dramatic reduction in mGluR5 plasma membrane expression 24 hours post-cocaine injection in mouse accumbal neurons. The use of different species, approaches and experimental conditions between this study and ours may account for these divergent results. Although the limited sensitivity of the immunogold method to localize small amount of protein cannot be ruled out (Galvan et al., 2006), it is noteworthy that the same method was sensitive enough to detect changes in both group I mGluRs plasma membrane expression following intra-accumbens DHPG administration, and was successfully used by different groups to demonstrate internalization

of various GPCRs in response to systemic or local agonist application (Bernard et al., 1998; Dumartin et al., 1998; Bernard et al., 1999; Csaba et al., 2001).

In striking contrast with group I mGluRs, AMPA receptor subunits display significant and opposite changes in their subsynaptic distribution after acute or chronic cocaine exposures (Boudreau and Wolf, 2005; Boudreau et al., 2007). Following the chronic behavioral sensitization paradigm, known to decrease extracellular glutamate (Pierce et al., 1996; Baker et al., 2003), an increased surface expression of synaptic AMPA receptors was found in the rat accumbens compared to saline-treated animals (Boudreau and Wolf, 2005). In contrast, acute cocaine exposure, which results in a significant raise in accumbens glutamate (Smith et al., 1995; Reid et al., 1997), lead to the internalization of AMPA GluR1- and GluR2-containing receptors (Boudreau and Wolf, 2005; Boudreau et al., 2007). Although these findings likely illustrate genuine differences in the trafficking of AMPA versus group I mGluRs in response to cocaine-induced changes in extracellular glutamate, the use of different techniques measure the surface expression of the receptors, may also account for these differential responses between ionotropic and metabotropic receptors after cocaine challenges. An important issue to consider is the exact localization of these glutamate receptors in relation to the release sites of glutamate. In contrast to AMPA receptor subunits, which are mainly concentrated in the main body of asymmetric glutamatergic synapses (Petralia et al., 1992; Baude et al., 1995), both mGluR1a and mGluR5 are largely extrasynaptic or perisynaptic to glutamatergic synapses (Baude et al., 1993; Ottersen and Landsend, 1997; Galvan et al., 2006). In light of data showing that increased glutamatergic transmission at cortical synapses may account for the raise in extracellular glutamate measured in the accumbens after acute cocaine administration (McFarland et al., 2003), the rapid internalization of AMPA receptors following such treatment is predictable because of the increase of glutamate in the synaptic cleft. On the other hand, group I mGluRs, located further away from these synapses may be less affected by this synaptically regulated change in glutamate release. Although this may partly account for the differential responses of these receptors after acute cocaine, it is unlikely to be the case in chronically treated animals which display a significant reduction in extracellular glutamate mainly due to functional changes in the cystine/glutamate exchangers predominantly located on glial cells (Baker et al., 2003). Overall, these findings indicate that cocaine can induce significant changes in AMPA receptor subsynaptic localization, but not as much in group I mGluRs. The mechanisms underlying these differential effects may be complex and involve a multitude of factors including the localization, trafficking as well as the pharmacological and physiological properties of these receptors.

A few *in vivo* studies demonstrated significant changes in the ultrastructural localization of GPCRs in response to modulation of extracellular activating neurotransmitter (Dumartin et al., 1998; Dumartin et al., 2000; Bernard et al., 2003; Toda et al., 2003; Stanwood and Levitt, 2007). However, in MPTP-treated mice, an experimental condition characterized by a significant increase in glutamatergic transmission in the subthalamic nucleus and globus pallidus (Mally et al., 1997; Fedele et al., 2001; Wichmann and DeLong, 2003), very little, if any, changes were induced in the subsynaptic and subcellular distribution of group I mGluRs in these nuclei (Kuwajima et al., 2007). Together, these findings highlight the unique intrinsic properties of group I mGluRs compared to other GPCRs in their response to changes in ambient activating transmitter levels. Receptor phosphorylation, lateral trafficking and receptor-receptor interactions are additional mechanisms, not examined in this study, that should be considered as playing major roles in regulating group I mGluRs responses to agonist exposure (Bernard et al., 2006; Dhami et al., 2006).

In contrast to systemic cocaine, intracerebral injections of group I mGluR agonist significantly decreased the percentage of plasma membrane-bound mGluR1a and mGluR5 in the accumbens. These observations are consistent with previous studies of other GPCRs known

to internalize following direct agonist application (Bernard et al., 1998; ; Dumartin et al., 1998; Bernard et al., 1999; Csaba et al., 2001). Together, these *in vivo* data demonstrate that group I mGluRs share common internalization properties with other GPCRs when directly exposed to high dose of receptor agonists, but not in response to physiological or pathological changes in the level of ambient activating neurotransmitter. One possible explanation might be that the magnitude of cocaine-induced changes in glutamate release is not high enough to affect group I mGluRs trafficking. Another possibility is that the mechanism underlying the internalization of group I mGluRs after DHPG is different from that following glutamate-mediated receptor activation. For instance, *in vitro* data have shown that application of glutamate versus the agonist quisqualate attract different arrestin proteins that aid in internalization of mGluR1a (Dale et al., 2001; Mundell et al., 2001). Thus, the mechanisms underlying group I mGluRs internalization appear to be quite complex and highly dependent on various factors including the source and pharmacological properties of the activating agonist.

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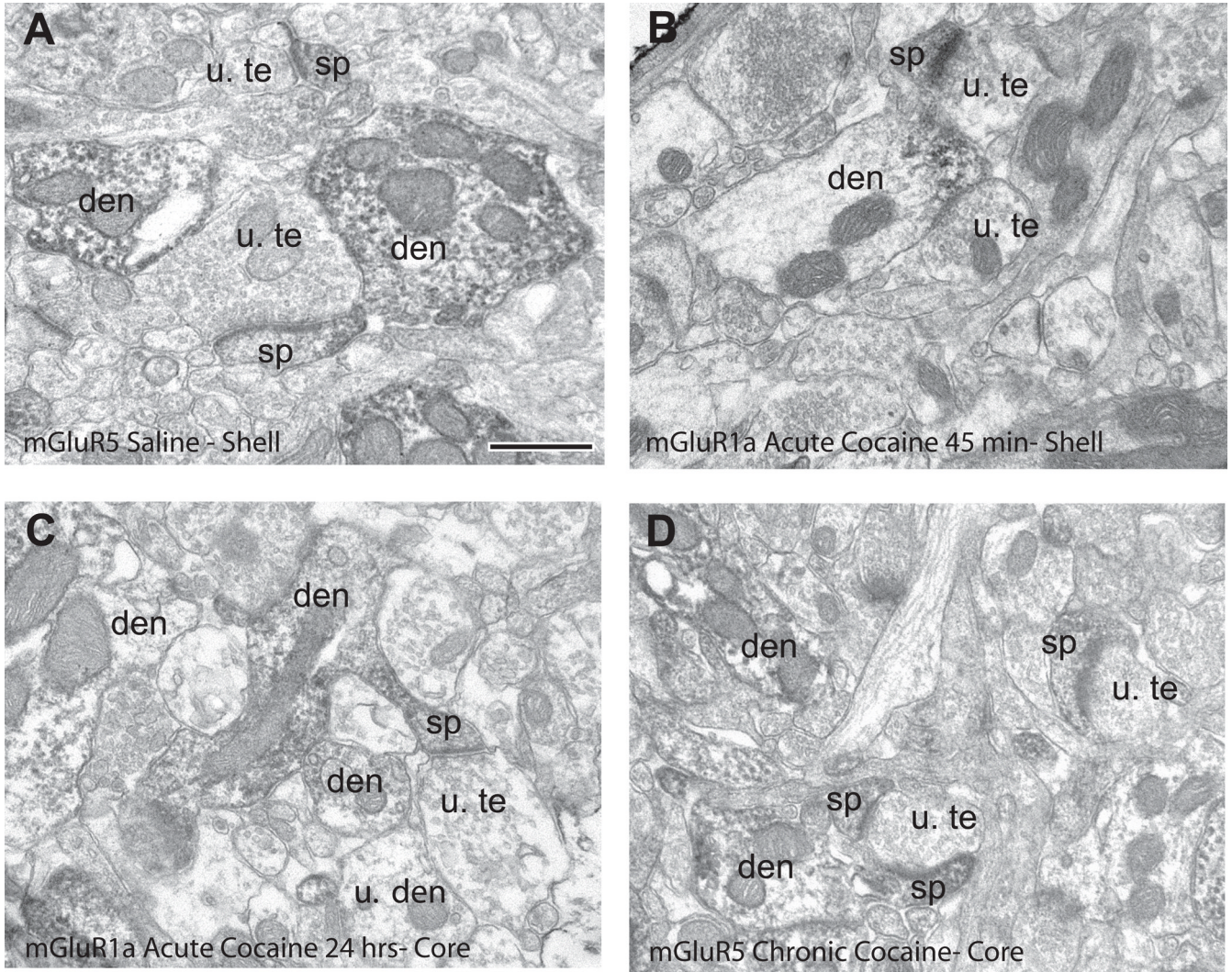
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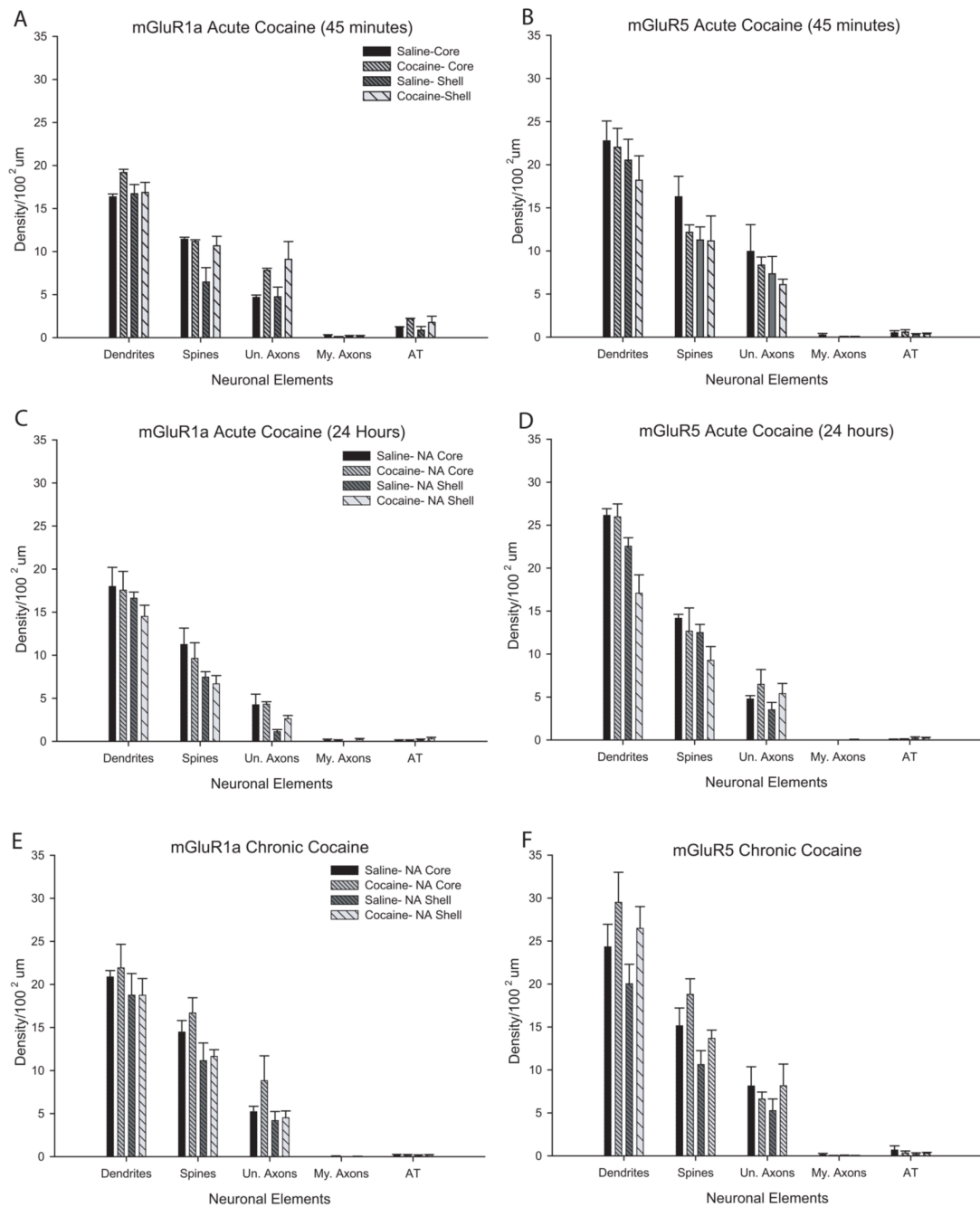
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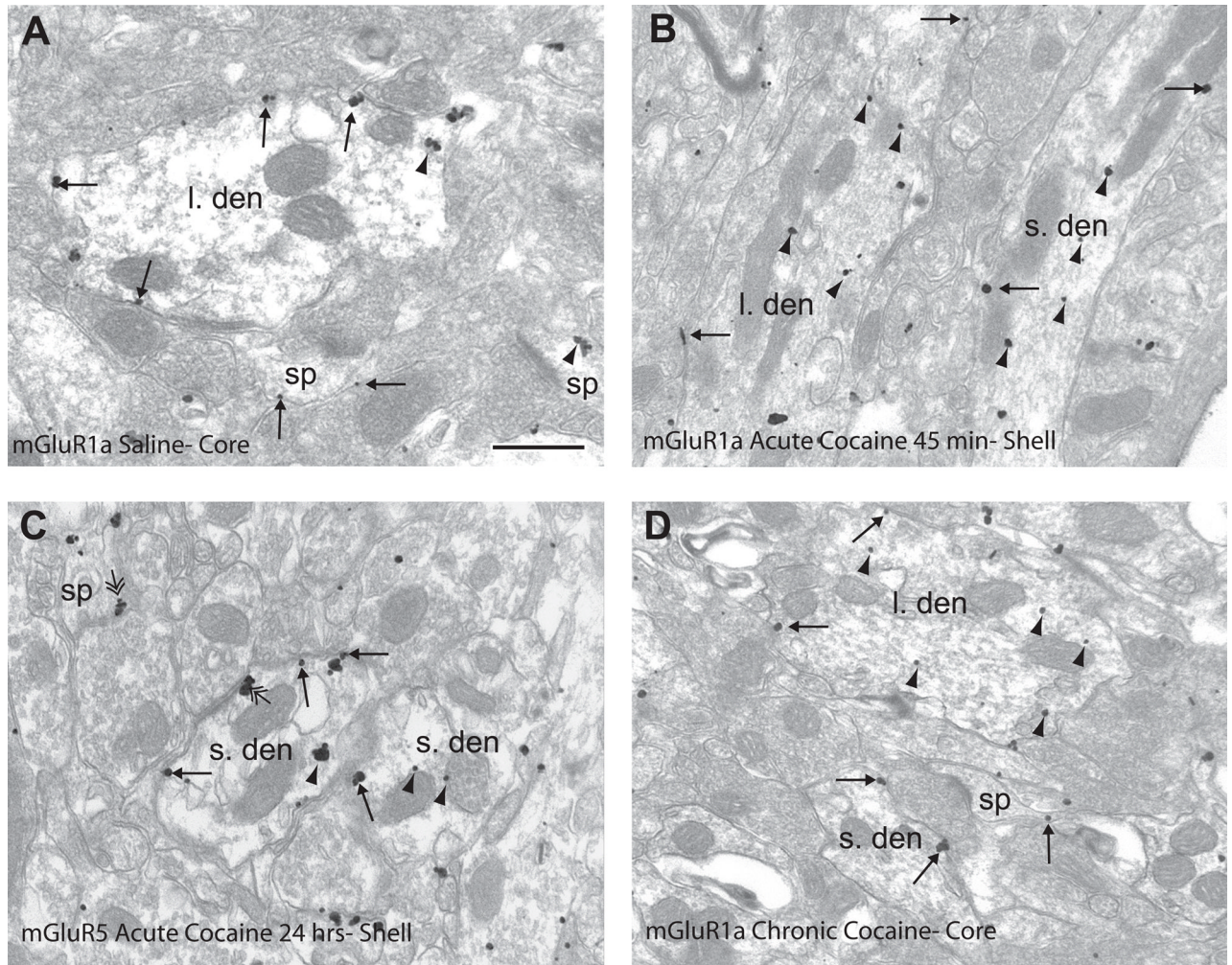
**Figure 1.** Immunoperoxidase labeling of mGluR1a and mGluR5 in saline- and cocaine-treated rats. (A) mGluR5-labeled elements in the nucleus accumbens shell of a saline-treated rat. (B) mGluR1a-labeled elements in the nucleus accumbens shell of a rat treated with cocaine, 45 minutes withdrawal. (C) mGluR1a-labeled elements in the nucleus accumbens core of a rat treated with cocaine, 24 hours withdrawal. (D) mGluR5-labeled elements in the nucleus accumbens core of animals treated chronically with cocaine for one week. Note the bulk of postsynaptic labeling in dendrites (den) and spines (sp). Abbreviations: u. te, unlabeled terminal; u. den, unlabeled dendrite; u. sp, unlabeled spine. Scale bar = 0.5  $\mu$ m.



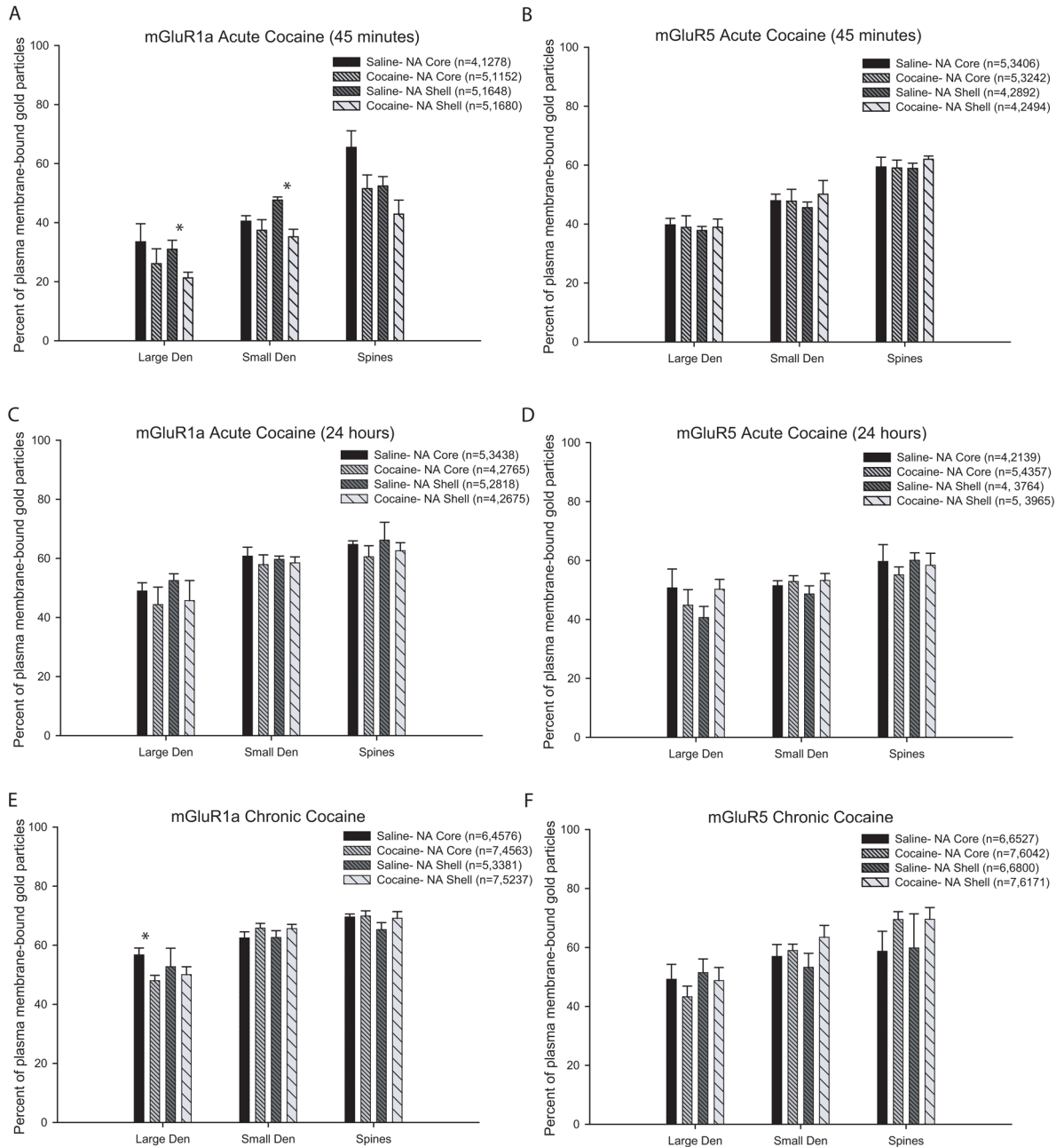
**Figure 2.** Histograms summarizing the distribution of mGluR1a and mGluR5 immunoperoxidase labeling in saline- and cocaine-treated rats, expressed as mean density of labeled elements per  $100\mu\text{m}^2$  ( $\pm$ SEM). (A–B) Immunoperoxidase labeling for mGluR1a and mGluR5 in the core and shell of the nucleus accumbens 45 minutes following a saline or cocaine injection. Total number of animals and labeled elements: mGluR1a: saline, core=4, 552; saline, shell=4, 470; cocaine, core=5, 822; cocaine, shell=5, 784. mGluR5: saline, core=5, 1012; saline, shell=5, 807; cocaine, core=5, 882; cocaine, shell=5, 729. (C–D) Immunoperoxidase labeling for mGluR1a and mGluR5 24 hours following saline or cocaine. Total number of animals and labeled elements: mGluR1a: saline, core=5, 786; saline, shell=4, 473; cocaine, core=5, 672;



cocaine, shell=5, 643. mGluR5: saline, core=4, 831; saline, shell=4, 710; cocaine, core=5, 1053; cocaine, shell=5, 694. (E-F) Immunoperoxidase labeling for mGluR1a and mGluR5 3 weeks following one-week chronic saline or cocaine. Total number of animals and labeled elements: mGluR1a: saline, core=7, 1682; saline, shell=7, 1381; cocaine, core=7, 1770; cocaine, shell=7, 1365. mGluR5: saline, core=7, 1897; saline, shell=7, 1471; cocaine, core=7, 1939; cocaine, shell=7, 1882. Abbreviations: Un. Axons: Unmyelinated axons; My. Axons: Myelinated axons; AT: Axon terminals.

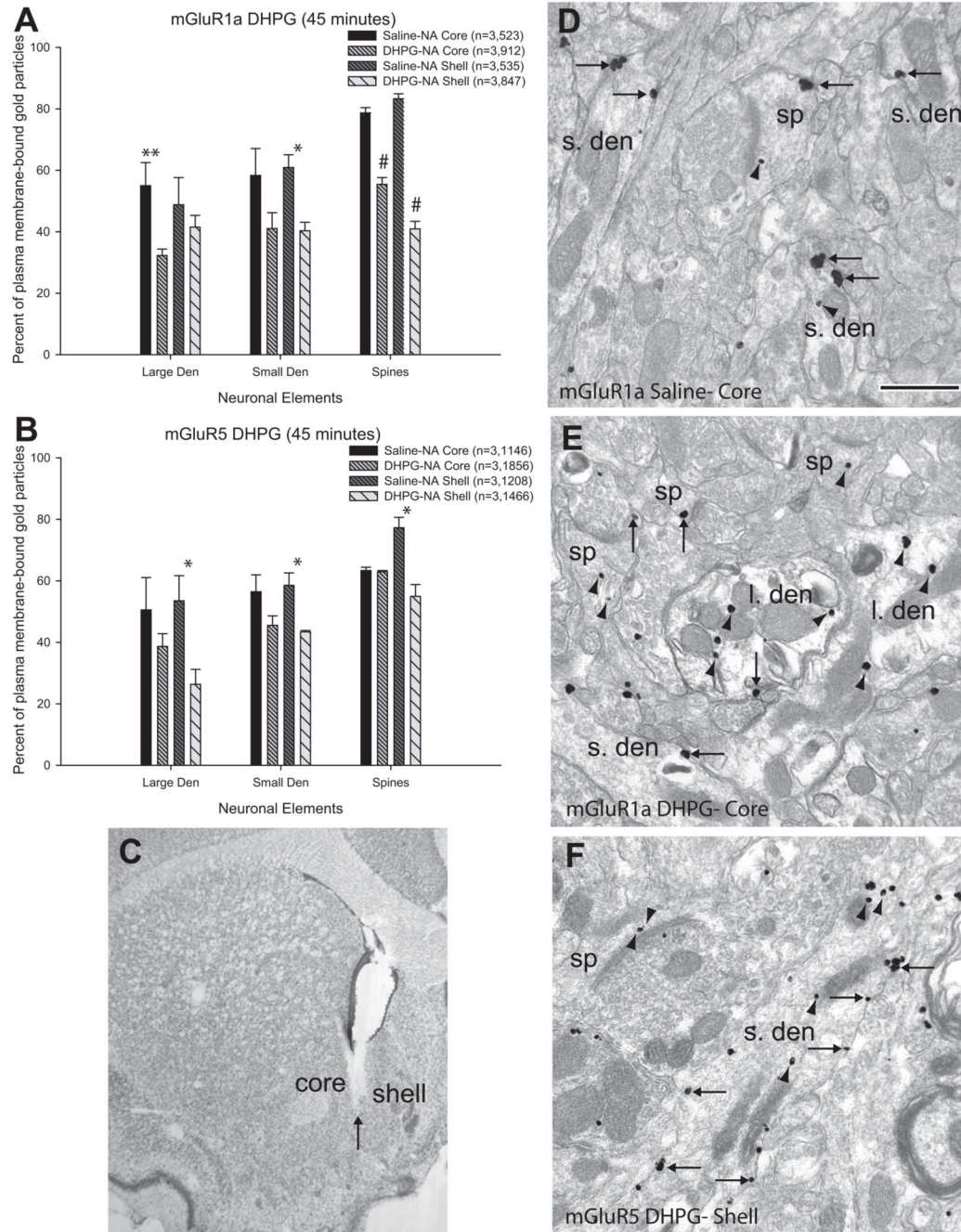


**Figure 3.** mGluR1a and mGluR5 immunogold labeling in saline- and cocaine-treated rats. (A) mGluR1a immunogold labeling in the nucleus accumbens core of a saline-treated rat. Note the extrasynaptic (single-headed arrows) labeling on the plasma membrane of the large dendrite (l. den) and spine (sp). (B) mGluR1a immunogold labeling in nucleus accumbens shell 45 minutes following cocaine treatment. Note the increased intracellular labeling (arrowheads) in large and small dendrites (s. den). (C) mGluR5 immunogold labeling in the nucleus accumbens shell 24 hours following cocaine injection. mGluR5 is distributed extrasynaptically (single-headed arrows) and intracellularly (arrowheads) in the dendrites. Also note the perisynaptic labeling to an asymmetric axodendritic and axospinous synapse (double-headed arrow). (D) mGluR1a immunogold labeling in the nucleus accumbens core of a rat chronically treated with cocaine followed by 3 weeks withdrawal. Note the larger amount of intracellular labeling in the large dendrite and extrasyaptic plasma membrane labeling in the small dendrite and spine. Scale bar=0.5µm.

**Figure 4.**

Histograms showing the percentage of plasma membrane labeling of mGluR1a and mGluR5 in the core and shell of the nucleus accumbens of saline- and cocaine-treated rats. Data is presented as the mean percentage ( $\pm$ SEM) of gold particles on the plasma membrane of large or small dendrites and spines; in parentheses n= number of animals used followed by the total number of gold particles counted in each experimental group. (A–B) Mean percentage of plasma membrane-bound mGluR1a (A) and mGluR5 (B) labeling in rats sacrificed 45 minutes following cocaine treatment. One-way ANOVA with Tukey's post-hoc test revealed a lower proportion of plasma membrane-bound mGluR1a in large and small dendrites in the shell of cocaine-treated rats ( $p < 0.05$ , single asterisks). Total number of elements examined: mGluR1a: Saline, Core: large den=38, small den=233, spines=117; Cocaine, Core: large

den=55, small den=198, spines=114; Saline, Shell: large den=64, small den=236, spines=116; Cocaine, Shell: large den=52, small den=260, spines=140. mGluR5: Saline core: large den=64, small den=365, spines=248; Cocaine, Core: large den=44, small den=365, spines=248; Saline, Shell: large den=65, small den=260, spines=171; Cocaine, Shell: large den=47, small den=292, spines=170. (C–D) Percentage of plasma membrane-bound mGluR1a (C) and mGluR5 (D) in rats sacrificed 24 hours following cocaine treatment. No significant difference was found between the saline- and cocaine-treated animals. Total number of elements examined: mGluR1a: Saline, Core: large den=53, small den=432, spines=263; Cocaine, Core: large den=19, small den=342, spines=210; Saline, Shell: large den=34, small den=368, spines=216; Cocaine, Shell: large den=36, small den=311, spines=179. mGluR5: Saline, core: large den=20, small den=270, spines=165; Cocaine, Core: large den=40, small den=464, spines=315; Saline, Shell: large den=34, small den=416, spines=256; Cocaine, Shell: large den=39, small den=446, spines=263. (E–F) Percentage of plasma membrane-bound mGluR1a (E) and mGluR5 (F) in rats chronically treated with cocaine and sacrificed 3 weeks later. One-way ANOVA and Tukey's post-hoc tests revealed that there is significantly lower percentage of plasma membrane-bound mGluR1a in the accumbens core of rats treated chronically with cocaine compared to saline ( $p < 0.05$ , single asterisk). Total number of elements examined: mGluR1a: Saline, Core: large den=53, small den=575, spines=380; Cocaine, Core: large den=76, small den=667, spines=531; Saline, Shell: large den=53, small den=427, spines=264; Cocaine, Shell: large den=83, small den=732, spines=427. mGluR5: Saline, core: large den=78, small den=717, spines=430; Cocaine, Core: large den=85, small den=748, spines=471; Saline, Shell: large den=104, small den=678, spines=372; Cocaine, Shell: large den=71, small den=783, spines=427.



**Figure 5.**

Histograms and immunogold labeling for mGluR1a and mGluR5 in saline- and DHPG-treated rats. (A) and (B) are summary histograms showing the percentage of plasma membrane-bound labeling for mGluR1a (A) and mGluR5 (B) in the core and shell of the nucleus accumbens of saline- and DHPG-treated rats. Data are presented as mean percentages ( $\pm$ SEM) of gold particles on the plasma membrane of large or small dendrites and spines; in parentheses n= number of animals used, followed by the total number of gold particles counted in each experimental group. One-way ANOVAs and Tukey's post-hoc tests reveal that there is a lower percentage of plasma membrane-bound mGluR1a in large dendrites of the core ( $p < 0.05$ , double asterisk), in small dendrites of the shell ( $p < 0.05$ , single asterisk), and in spines of both shell

and core of the accumbens ( $p < 0.001$ , number signs) in DHPG-treated animals. For mGluR5, there is a significantly lower percentage of labeling on the plasma membrane in the shell of the accumbens on large and small dendrites ( $p < 0.05$ , single asterisk) and spines ( $p < 0.01$ , number sign). (C) Light micrograph of a sample DHPG injection into the core and medial shell of the nucleus accumbens. The arrow indicates the tip of the syringe. (D–F) show examples of labeled elements from saline-treated (D) and DHPG-treated animals (E–F). (D) mGluR1a labeling in the core of the accumbens of a saline-treated animal. Note the majority of extrasynaptic labeling (single-headed arrows) on the plasma membrane of small dendrites (s.den) and spines (sp). (E) mGluR1a labeling in the core of the accumbens of a DHPG-treated animal. Notice the increase in intracellular (arrowheads) labeling in both dendrites and spines. (F) mGluR5 labeling in the shell of the accumbens of a DHPG-treated animal. Note the large pool of intracellular labeling in dendrites and spines. Scale bar=0.5 $\mu$ m. Total number of elements examined: mGluR1a: Saline, Core: large den=15, small den=137, spines=72; DHPG, Core: large den=57, small den=170, spines=91; Saline, Shell: large den=24, small den=94, spines=40; DHPG, Shell: large den=50, small den=134, spines=60. mGluR5: Saline core: large den=36, small den=115, spines=111; DHPG, Core: large den=38, small den=190, spines=105; Saline, Shell: large den=34, small den=147, spines=47; DHPG, Shell: large den=32, small den=157, spines=57.

Subsynaptic group I mGluRs labeling after cocaine treatment in the accumbens core. Data are presented as percent of gold particles  $\pm$ SEM for each subsynaptic domain in Spines, Sm. Den (small dendrites) and Lg. Den (large dendrites). The different categories of subsynaptic domains are abbreviated as follows: Extra= extrasynaptic; Peri-Asym=perisynaptic labeling to asymmetric synapses; Syn-Asym=synaptic labeling of asymmetric synapses; Peri-Symm=perisynaptic labeling at symmetric synapses; Syn-Symm=synaptic labeling at symmetric synapses. Data for mGluR1a are in the top row of each box, while mGluR5 data are in the bottom row of each box. The number of animals used, elements examined and gold particles counted is presented in the legend of figure 4.

Table 1

	Acute Cocaine-45 MINUTES		Acute Cocaine-24 HOURS		Chronic Cocaine	
	Saline-Core	Cocaine-Core	Saline-Core	Cocaine-Core	Saline-Core	Cocaine-Core
<i>mGluR1a</i>						
<i>mGluR5</i>						
<i>SPINES</i> Extra	78.7 $\pm$ 4.5	79.8 $\pm$ 6.1	85.3 $\pm$ 2.3	81.0 $\pm$ 1.2	82.6 $\pm$ 1.3	86.8 $\pm$ 1.5
	84.3 $\pm$ 3.5	83.8 $\pm$ 2.1	82.7 $\pm$ 7.4	83.2 $\pm$ 2.2	83.1 $\pm$ 1.3	82.2 $\pm$ 1.3
Peri-Asym	18.3 $\pm$ 3.4	20.2 $\pm$ 6.1	14.5 $\pm$ 2.1	18.2 $\pm$ 1.4	14.8 $\pm$ 0.7	12.5 $\pm$ 1.4
	14.9 $\pm$ 1.7	16.0 $\pm$ 2.1	18.9 $\pm$ 5.5	14.8 $\pm$ 2.3	14.6 $\pm$ 1.9	16.2 $\pm$ 1.7
Syn-Asym	2.9 $\pm$ 1.4	0	0.3 $\pm$ 0.3	0.2 $\pm$ 0.2	1.6 $\pm$ 0.7	0.7 $\pm$ 0.3
	0.8 $\pm$ 0.6	0.2 $\pm$ 0.2	1.4 $\pm$ 0.7	1.7 $\pm$ 0.5	1.1 $\pm$ 0.3	1.1 $\pm$ 0.4
Peri-Symm	0	0	0	0.6 $\pm$ 0.6	1.3 $\pm$ 0.6	0
	0	0	0.4 $\pm$ 0.4	0.3 $\pm$ 0.3	1.1 $\pm$ 0.4	0
Syn-Symm	0	0	0	0	0	0
	0	0	0	0	0.2 $\pm$ 0.2	0.3 $\pm$ 0.3
<i>SM. DEN</i> Extra	98.1 $\pm$ 1.2	96.3 $\pm$ 1.7	97.6 $\pm$ 0.8	96.2 $\pm$ 0.7	97.3 $\pm$ 0.9	98.5 $\pm$ 1.0
	98.5 $\pm$ 0.5	97.3 $\pm$ 1.4	97.9 $\pm$ 0.7	98.3 $\pm$ 0.6	98.5 $\pm$ 0.6	98.0 $\pm$ 0.4
Peri-Asym	0.3 $\pm$ 0.3	1.8 $\pm$ 1.2	1.1 $\pm$ 0.5	0.7 $\pm$ 0.3	0.9 $\pm$ 0.2	1.5 $\pm$ 0.2
	0.4 $\pm$ 0.2	0.7 $\pm$ 0.3	1.3 $\pm$ 0.7	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2	0.9 $\pm$ 0.4
Syn-Asym	0.3 $\pm$ 0.3	0	0.2 $\pm$ 0.1	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1	0 $\pm$ 0.1
	0.1 $\pm$ 0.1	0.3 $\pm$ 0.3	0.4 $\pm$ 0.4	0	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1
Peri-Symm	1.4 $\pm$ 0.8	1.9 $\pm$ 1.1	0.7 $\pm$ 0.3	1.3 $\pm$ 0.4	1.7 $\pm$ 0.9	0 $\pm$ 0.6
	0.8 $\pm$ 0.3	1.3 $\pm$ 1.0	0.2 $\pm$ 0.1	0.7 $\pm$ 0.3	0.7 $\pm$ 0.2	1.0 $\pm$ 0.3
Syn-Symm	0	0	0.5 $\pm$ 0.2	1.6 $\pm$ 0.6	0.1 $\pm$ 0.1	1.0 $\pm$ 0.5
	0.2 $\pm$ 0.1	0.5 $\pm$ 0.5	0.1 $\pm$ 0.1	0.7 $\pm$ 0.3	0.1 $\pm$ 0.1	0.1 $\pm$ 0.0
<i>LG. DEN</i> Extra	97.5 $\pm$ 1.6	96.1 $\pm$ 2.9	96.4 $\pm$ 1.5	94.9 $\pm$ 3.4	96.0 $\pm$ 1.5	96.5 $\pm$ 1.7
	94.2 $\pm$ 3.3	97.3 $\pm$ 1.7	100 $\pm$ 0	96.4 $\pm$ 2.3	96.7 $\pm$ 2.3	96.1 $\pm$ 1.5
Peri-Asym	0	0	1.9 $\pm$ 1.2	2.4 $\pm$ 2.4	0	0
	1.4 $\pm$ 1.4	0.8 $\pm$ 0.8	0	1.4 $\pm$ 0.9	0.9 $\pm$ 0.9	2.2 $\pm$ 1.3
Syn-Asym	0	0	0.3 $\pm$ 0.3	0	0	0
	0	0	0	0	0	0
Peri-Symm	2.4 $\pm$ 1.5	2.2 $\pm$ 2.2	0.8 $\pm$ 0.8	1.6 $\pm$ 1.6	4.0 $\pm$ 1.5	3.0 $\pm$ 1.8
	1.8 $\pm$ 1.1	0.8 $\pm$ 0.8	0	0.4 $\pm$ 0.4	2.3 $\pm$ 2.3	1.8 $\pm$ 1.0
Syn-Symm	0	1.7 $\pm$ 1.0	0.7 $\pm$ 0.4	1.2 $\pm$ 1.2	0	0.5 $\pm$ 0.5
	1.8 $\pm$ 1.4	1.2 $\pm$ 1.2	0	1.9 $\pm$ 1.9	0	0

Subsynaptic group I mGluRs labeling after cocaine treatment in the accumbens shell. Data are presented as percent of gold particles  $\pm$ SEM for each subsynaptic domain in Spines, Sm. Den (small dendrites) and Lg. Den (large dendrites). The different categories of subsynaptic domains are abbreviated as follows: Extra=extrasympaptic; Peri-Asym=perisynaptic labeling to asymmetric synapses; Syn-Asym=synaptic labeling of asymmetric synapses; Peri-Symm=perisynaptic labeling at symmetric synapses; Syn-Symm=synaptic labeling at symmetric synapses. Data for mGluR1a are in the top row of each box, while mGluR5 data are in the bottom row of each box. The number of animals used, elements examined and gold particles counted is presented in the legend of figure 4.

Table 2

	Acute Cocaine-45 MINUTES		Acute Cocaine-24 HOURS		Chronic Cocaine	
	Saline-Shell	Cocaine-Shell	Saline-Shell	Cocaine-Shell	Saline-Shell	Cocaine-Shell
<i>mGluR1a</i>						
<i>mGluR5</i>						
<b>SPINES</b> Extra	79.5 $\pm$ 4.1	73.8 $\pm$ 1.7	83.7 $\pm$ 2.8	85.6 $\pm$ 1.9	82.2 $\pm$ 3.3	85.5 $\pm$ 2.4
Peri-Asym	82.6 $\pm$ 3.5	85.4 $\pm$ 3.6	88.0 $\pm$ 1.7	85.3 $\pm$ 2.5	80.6 $\pm$ 1.3	84.3 $\pm$ 1.4
Syn-Asym	16.6 $\pm$ 4.5	21.0 $\pm$ 1.2	13.3 $\pm$ 1.3	13.5 $\pm$ 1.5	14.7 $\pm$ 2.4	13.0 $\pm$ 2.2
Peri-Symm	17.1 $\pm$ 3.4	13.6 $\pm$ 3.2	10.9 $\pm$ 1.4	13.0 $\pm$ 2.0	17.7 $\pm$ 1.5	13.6 $\pm$ 1.6
Syn-Symm	3.9 $\pm$ 1.1	5.2 $\pm$ 2.6	2.0 $\pm$ 1.1	0.5 $\pm$ 0.6	1.0 $\pm$ 0.6	0.9 $\pm$ 0.3
Extra	0.3 $\pm$ 0.3	1.0 $\pm$ 0.6	1.1 $\pm$ 0.7	1.3 $\pm$ 0.8	1.3 $\pm$ 0.5	1.6 $\pm$ 0.6
SM. DEN	0	0	0.8 $\pm$ 0.8	0.5 $\pm$ 0.5	1.7 $\pm$ 1.4	0.6 $\pm$ 0.5
Peri-Asym	0	0	0	0.2 $\pm$ 0.2	0.4 $\pm$ 0.4	0.6 $\pm$ 0.4
Syn-Asym	0	0	0.3 $\pm$ 0.3	0	0.5 $\pm$ 0.3	0
Peri-Symm	0	0	0	0.2 $\pm$ 0.2	0	0
Syn-Symm	0	0	0	0	0	0
<b>SM. DEN</b> Extra	94.7 $\pm$ 1.7	93.0 $\pm$ 1.5	96.8 $\pm$ 0.5	96.3 $\pm$ 0.4	96.4 $\pm$ 1.2	95.4 $\pm$ 0.9
Peri-Asym	97.0 $\pm$ 1.0	98.0 $\pm$ 1.0	98.3 $\pm$ 0.5	97.9 $\pm$ 0.5	97.9 $\pm$ 0.6	95.9 $\pm$ 0.9
Syn-Asym	2.3 $\pm$ 1.0	2.6 $\pm$ 0.9	1.6 $\pm$ 0.5	0.7 $\pm$ 0.4	1.2 $\pm$ 0.5	2.0 $\pm$ 0.6
Peri-Symm	1.9 $\pm$ 0.5	0.6 $\pm$ 0.4	0.5 $\pm$ 0.3	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2	1.1 $\pm$ 0.4
Syn-Symm	0.4 $\pm$ 0.4	0	0.2 $\pm$ 0.2	0.1 $\pm$ 0.1	0	0
Extra	0.1 $\pm$ 0.1	0.3 $\pm$ 0.2	0	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0
SM. DEN	1.4 $\pm$ 0.6	2.1 $\pm$ 0.9	0.1 $\pm$ 0.1	1.9 $\pm$ 0.4	1.5 $\pm$ 0.8	2.3 $\pm$ 0.6
Peri-Asym	0.7 $\pm$ 0.4	0.5 $\pm$ 0.5	0.9 $\pm$ 0.4	0.9 $\pm$ 0.4	1.0 $\pm$ 0.6	2.1 $\pm$ 0.6
Syn-Asym	1.2 $\pm$ 0.5	2.2 $\pm$ 1.0	1.4 $\pm$ 0.5	1.1 $\pm$ 0.3	0.8 $\pm$ 0.2	0.3 $\pm$ 0.2
Peri-Symm	0.2 $\pm$ 0.2	0.7 $\pm$ 0.4	0.3 $\pm$ 0.1	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.7 $\pm$ 0.1
Syn-Symm	100 $\pm$ 0	100 $\pm$ 0	98.2 $\pm$ 1.1	93.2 $\pm$ 1.9	88.8 $\pm$ 4.4	96.9 $\pm$ 1.8
<b>Lg. DEN</b> Extra	98.0 $\pm$ 1.2	99.5 $\pm$ 0.5	97.4 $\pm$ 2.6	98.5 $\pm$ 1.5	98.4 $\pm$ 1.0	93.6 $\pm$ 2.1
Peri-Asym	0	0	0	1.6 $\pm$ 1.6	4.9 $\pm$ 1.8	0.1 $\pm$ 0.1
Syn-Asym	0.6 $\pm$ 0.6	0	0	0	0.8 $\pm$ 0.6	0.3 $\pm$ 0.3
Peri-Symm	0	0	0.9 $\pm$ 0.9	0	2.2 $\pm$ 2.2	0.3 $\pm$ 0.3
Syn-Symm	0	0	0.5 $\pm$ 0.5	0	0	0
Extra	0	0	0.6 $\pm$ 0.6	5.3 $\pm$ 0.5	2.5 $\pm$ 1.6	2.4 $\pm$ 1.6
SM. DEN	1.3 $\pm$ 0.8	0	1.6 $\pm$ 1.6	1.0 $\pm$ 1.0	0.7 $\pm$ 0.7	2.7 $\pm$ 1.4
Peri-Asym	0	0	0.3 $\pm$ 0.3	0	1.6 $\pm$ 1.6	0.2 $\pm$ 0.2
Syn-Asym	0	0.5 $\pm$ 0.5	0.5 $\pm$ 0.5	0.5 $\pm$ 0.5	0.1 $\pm$ 0.1	3.4 $\pm$ 2.4