Published in final edited form as:

Neuropharmacology. 2011 December ; 61(7): 1129–1140. doi:10.1016/j.neuropharm.2010.12.031.

Differential glutamate AMPA-receptor plasticity in subpopulations of VTA neurons in the presence or absence of residual cocaine: Implications for the development of addiction

D.A. Lane^a, B. Reed^b, M.J. Kreek^b, and V.M. Pickel^a

^a Dept. of Neurology and Neuroscience, Weill Medical College of Cornell University

^b Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY 10065

Abstract

Cocaine-induced plasticity of mesocorticolimbic dopamine (DA) neurons, originating in the ventral tegmental area (VTA), persists in the absence of cocaine and may contribute to both drug-craving and relapse. Glutamate AMPA receptors (AMPA) in these neurons are implicated in this plasticity. However, there is no ultrastructural evidence that the absence of cocaine following repeated administrations affects the critical surface/synaptic availability of AMPAR GluR1 subunits in either DA or non-DA, putative GABAergic neurons within the VTA. To assess this, we used electron microscopic immunolabeling in the VTA of adult male mice sacrificed at 30 minutes or 72 hours after receiving the final of six (15 mg/kg) cocaine injections, a dosing paradigm that resulted in development of locomotor sensitization. At each time point, both cocaine- and saline-injected mice showed AMPAR GluR1 immunogold labeling in somatodendritic profiles, many of which contained immunoperoxidase labeling for the DA-synthesizing enzyme, tyrosine hydroxylase (TH). At 30 minutes after the last injection, when cocaine was systemically present, only the non-TH labeled dendrites showed a significant increase in the synaptic/plasmalemmal density of GluR1 immunogold particles. At 72 hours, when systemic cocaine was depleted, synaptic GluR1 labeling was greatly enhanced in TH-containing dendrites throughout the VTA and in non-TH dendrites of the limbic-associated paranigral VTA. Our results demonstrate that systemic cocaine produces GluR1 trafficking specifically in non-DA neurons of the VTA, which may subsequently contribute to the abstinent-induced enhancement of AMPA receptor synaptic transmission in mesocorticolimbic DA neurons leading to heightened drug seeking behavior.

Keywords

dopamine; GABA; tyrosine hydroxylase; ultrastructure; multivesicular bodies; reward

Corresponding Author: Diane Lane, Ph.D., Department of Neurology and Neuroscience, Division of Neurobiology, Weill-Cornell Medical College, 407 E. 61st St, New York, NY 10065, dal2021@med.cornell.edu, Phone: (646) 962–8257.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Prior cocaine exposure produces a relatively permanent change in the function of the mesocorticolimbic dopamine system such that the absence of cocaine activates neuronal mechanisms aimed at increasing drug seeking behavior and the reinstatement of drug use. Decreasing forebrain dopamine levels after the cessation of cocaine self-administration leads to additional cocaine intake (Wise et al., 1995) and craving for cocaine increases over time during periods of abstinence (Grimm et al., 2001). The time course of these changes corresponds to the development of cocaine-induced locomotor sensitization suggesting that similar mechanisms may mediate both events (Grimm et al., 2001; Lu et al., 2004; Robinson and Berridge, 1993).

Increased AMPA receptor (AMPA) mediated activation of ventral tegmental area (VTA) dopaminergic neurons is associated with repeated cocaine administration, a mechanism thought to contribute to the development of addiction (Kalivas and Stewart, 1991; Kauer and Malenka, 2007; Kessels and Malinow, 2009; Wolf, 2003 for reviews). Support for this idea comes from studies showing that cocaine increases extracellular glutamate levels (Kalivas and Duffy, 1988) and responsiveness to AMPA administered directly into the VTA (White et al., 1995; Zhang et al., 1997). Furthermore, either single or repeated cocaine administration increases AMPA-mediated glutamatergic activation of dopaminergic neurons in the VTA (Bellone and Luscher, 2006; Saal, 2003; Ungless et al., 2001).

Functionally, increased AMPAR-mediated activation of VTA dopaminergic neurons results in a progressive increase in locomotor activity known as behavioral sensitization, an animal model which parallels neuroadaptations seen with addiction (Carlezon et al., 1997; Kalivas and Stewart, 1991) and drug craving (Robinson and Berridge, 1993). The involvement of VTA AMPARs in cocaine-induced locomotor sensitization is supported by several lines of evidence. First, injections of either cocaine (Cornish and Kalivas, 2001) or AMPA (Dunn et al., 2005) directly into the VTA result in an increase in locomotor activity following a subsequent systemic challenge-dose of cocaine. Second, animals with greater cocaine-induced locomotor activity show greater AMPAR-mediated activation of VTA dopaminergic neurons (Borgland et al., 2004). Conversely, intra-VTA administration of a glutamate receptor antagonist prevents the development of behavioral sensitization (Karler et al., 1989) and conditioned place preference (Harris and Aston-Jones, 2003) to cocaine. These studies demonstrate the functional importance of AMPAR activation of dopaminergic neurons in the VTA in the neuroplasticity associated with animal models of addiction.

There is considerable evidence that cocaine increases AMPAR GluR1 expression and activation of VTA dopaminergic neurons (Bellone and Luscher, 2006; Fitzgerald et al., 1996; Saal, 2003; Ungless et al., 2001). However, most studies examined cocaine-induced changes in AMPAR-mediated activation of VTA dopamine neurons long after cessation of cocaine administration when animals were in withdrawal, a time when VTA GluR1 levels are the greatest (Tang et al., 2004). Thus, the lack of cocaine, after prior exposure, may be a critical determinant of the changes in the expression and synaptic targeting of VTA GluR1-containing AMPARs associated with addiction to cocaine. To test this hypothesis, we examined the electron microscopic immunolabeling of AMPAR GluR1 subunits in the VTA at either 30 minutes or 72-hours after repeated cocaine administration in mice that displayed locomotor sensitization. Immunogold labeling of AMPAR GluR1 subunits was combined with immunoperoxidase localization of tyrosine hydroxylase (TH), a mandatory enzyme for dopamine synthesis not present in the many GABAergic neurons of the VTA (Bayer and Pickel, 1991). Our results provide evidence that prior cocaine exposure produces changes in the postsynaptic (dendritic) surface trafficking of GluR1-containing AMPARs in non-

dopamine and dopamine neurons of the VTA depending on the respective presence or absence of cocaine.

2. Methods

2.1 Subjects

Thirty naïve adult male C57/BL6 mice (Jackson Labs, Bar Harbor, ME, USA), weighing 21–27 g (starting weight) were individually housed and maintained on a 12-hour light cycle (lights out at 6:00 pm). Food and water were available *ad libitum*. Experimental protocols involving animals followed NIH guidelines to the care and use of research animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at Weill Cornell Medical College.

2.2 Cocaine Administration and Locomotor testing Paradigm

All mice were randomly assigned to one of three treatment groups: repeated cocaine sacrificed 30 minutes after the final cocaine injection (30 min), repeated cocaine sacrificed 72 hours after the final cocaine injection (72 hr), or saline controls (Sal; Figure 1). One day prior to commencement of drug injection trials, baseline levels of locomotor activity were measured for 30 minutes in open-field test chambers (Med Associates, St. Albans, VT). For the next 5 consecutive days, all mice received one subcutaneous (s.c.) injection per day of either 15 mg/kg cocaine or equal volume of saline. Locomotor activity was measured for 30 minutes immediately after each injection. The mice received no injections or behavioral testing for the next 6 consecutive days. On Day 12, mice were given one final injection of cocaine (15 mg/kg) or saline and locomotor activity was measured for 30 minutes to assess potential duration of sensitized behavior. Immediately after locomotor testing on Day 12, 10 cocaine-treated mice were processed for blood-plasma cocaine detection (n=4; see section 2.3) or electron microscopic immunocytochemistry (n= 6; see sections 2.4 and 2.6 below). The remaining 20 mice continued behavioral testing until Day 15 (72 hours after the final cocaine injection). On Day 15 the mice received no injections and locomotor activity was measured for 30 minutes. Immediately after behavioral testing tissue was processed for HPLC analysis of plasma cocaine levels (n=4; cocaine-treated mice only) or electron microscopic immunocytochemistry (n=12; cocaine and saline-treated mice) to assess the subcellular distribution of AMPAR GluR1 subunit labeling in VTA neurons in the presence and absence of cocaine.

2.3 Measurement of Plasma Cocaine and Benzoyllecgonine Levels

Plasma preparation and HPLC were performed in similar fashion to that used by Benuck and colleagues (Benuck et al., 1987) for the detection of cocaine and cocaine metabolite levels in mouse plasma at 30 minutes or 72 hours after final cocaine injection. Plasma was collected via direct arterial sampling and placed in EDTA tubes on ice. Plasma was obtained via centrifugation (3000 × g, 10 min., 4°C), followed by addition of 2.5 µl NaF/100 µl plasma. Two micrograms of lidocaine (in methanol) was added as internal standard. To 100 µl plasma, 300 µl acetonitrile was added, followed by centrifugation (13000 × g, 2 min.). To the supernatant, 700 µl 80:20 chloroform: ethanol was added, followed by 70 µl NaHCO₃. Samples were vortexed for 30 seconds, followed by centrifugation (13000 × g, 2 min). The aqueous phase was discarded, and samples were dried under a stream of nitrogen. Dried samples were resuspended in 60 µl 50% MeOH, followed by analysis using HPLC-uv-vis detection.

The HPLC system used was a Series 1100 binary pump and diode array detector. A Supelco LC-ABZ (Bellefonte, PA, USA) column, 4.6mm × 15 cm, was used, with a mobile phase consisting of 75% 0.1M monobasic potassium phosphate (pH 6.7), 25% acetonitrile, with a

flow rate of 1300 µl/minute. The diode array detector detects the full spectrum of absorbance of the HPLC eluate; absorbance at 235 nm was used for quantification. Standard curves were generated by the addition of benzoylecgonine and cocaine to plasma from naïve animals covering the concentration range of 50–5000 ng/ml. The retention time for benzoylecgonine in our system is 2.4 minutes, that for cocaine is 4.3 minutes. The limit of quantification for both cocaine and benzoylecgonine was approximately 50 ng/ml.

2.4 Tissue Preparation for Immunocytochemistry

All mice were deeply anesthetized with sodium pentobarbital (120 mg/kg, i.p.; Sigma, St. Louis, MO) and perfused through the right ventricle with 20 ml heparin (1,000 U/ml) in saline (American Pharmaceutical Partners, Schaumburg, IL) followed by 50 ml of 3.75% acrolein (Polysciences, Warrington, PA) in 2% paraformaldehyde in 0.1 M phosphate buffer solution and finally 100 ml of 2% paraformaldehyde in 0.1 M phosphate buffer solution (Sigma, St. Louis, MO). The brains were removed from the cranium and cut into 3–4 mm coronal blocks that were postfixed in 2% paraformaldehyde for 30 minutes, then sliced to 40 µm sections on a Leica Vibratome VT1000 (Leica Instruments, Nussloch, Germany) in chilled 0.1 M phosphate buffer solution. The collected vibratome sections were placed in a storage solution (30% sucrose, 30% ethylene glycol in 0.05 M PB, pH 7.4) at –20°C until used for immunolabeling of GluR1 and/or TH.

2.5 Antisera

GluR1 immunolabeling was achieved using an affinity purified rabbit polyclonal antibody raised against a 15 amino acid peptide sequence corresponding to the C-terminus of rat GluR1 AMPA subunit (Chemicon, Temecula, CA). The GluR1 antiserum is well characterized by immunolabeling and Western blot analysis, which shows that this antiserum recognizes a single band of 110 kDa corresponding to the GluR1 subunit with no cross-reaction with GluR2–4 subunits (Aicher et al., 2002; Glass et al., 2005; Siegel et al., 1995).

A mouse monoclonal antibody raised against the rate-limiting enzyme, tyrosine hydroxylase (TH), was commercially obtained from Immunostar (Hudson, WI). The TH immunogen was purified to homogeneity from PC12 cells of rat origin. Western blot analysis shows that this antiserum identifies a single 60 kDa band exclusively in TH transfected, but not non-transfected, HEK293 cells (Immunostar; see Lessard et al., 2010). Moreover, in brainstem catecholaminergic cell groups the TH-immunoreactivity detected using this antibody is comparable to the distribution of TH mRNA as seen by *in situ* hybridization (Rusnak and Gainer, 2005).

2.6 Immunocytochemistry

The dual-labeling immunocytochemistry protocol used in the present study has been previously described in detail (Lane et al., 2010; Lane et al., 2008). Coronal sections of tissue containing the VTA of both cocaine-injected experimental groups and saline-injected animals were co-processed using a dual immunogold-silver method for the detection of the antiserum against GluR1 subunit of the AMPA glutamate receptor and immunoperoxidase for detection of TH antibody. Co-processing eliminated potential variability in labeling between experimental groups. The pre-embedding dual-labeling protocol used in the present study was adapted from that of Chan and colleagues (Chan et al., 1990). This method optimally preserves the fine structural detail necessary for valid quantification of receptor proteins on extrasynaptic membranes that can be lost during the process of plastic embedding (Adams et al., 2002), while minimizing spurious attachment of gold particles often seen in plastic with post-embedding immunogold labeling (Leranth, 1989).

2.7 Selection and sectioning of VTA tissue

The VTA, containing both the parabrachial (PB) and paranigral (PN) subregions, was cut from the flat-embedded coronal sections of tissue. The PB VTA contains mesocortical dopaminergic neurons which primarily project to the medial prefrontal cortex (mPFC; Carr and Sesack, 2000b; Lane et al., 2008), a region important for drug seeking behavior and reinstatement of cocaine self-administration (Capriles et al., 2003; Goeders and Smith, 1983; McFarland and Kalivas, 2001; Park et al., 2002; Robbins, 1996; Sun et al., 2005). The PN VTA contains mesolimbic dopaminergic neurons with principal projections to the nucleus accumbens (NAc), a brain region important for mediating reward (and aversion) salience (Jentsch and Taylor, 1999). These regions were identified by comparing the distributions of TH-immunoreactivity in reference to landmark structures identified in a mouse brain atlas (Hof et al., 2000). A medial/caudal region of the VTA (ranging from -3.30 to -3.50 mm from Bregma) was selected based on the greatest density of TH-immunoreactive cells and the prevalence of both VTA regions within the same coronal plane. Precise regional selection helped to avoid changes in GluR1 distribution ascribed to differences in the ratio of dopaminergic and GABAergic neurons that exist throughout the rostral to caudal aspects of VTA (Olson and Nestler, 2007).

Ultrathin sections (70 nm) were cut from the selected VTA tissue using an ultratome (Leica Instruments, Nussloch, Germany) and a diamond knife (Diatome, Fort Washington, PA). These sections were obtained from the surface (1–2 μm) of the flat-embedded tissue, where there is optimal penetration of immunoreagents. The sections were collected on 400-mesh copper grids (Electron Microscopy Science, Fort Washington, PA), counterstained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Phillips CM-10 electron microscope (FEI, Hillsboro, OR). The microscopic images were captured with an AMT Advantage HR/HR-B CCD Camera System (Advanced Microscopy Techniques, Danvers MA).

2.8 Data Analysis

A total tissue area of $24,576 \mu\text{m}^2$ (1,132 micrographs at 19,000 to 25,000x magnification) was examined from the VTA of 18 mice in three treatment groups. Micrographs of the neuropil were collected throughout each region of the VTA to eliminate the possibility of biasing the data. In individual profiles, GluR1 immunogold labeling was evident as small punctuate electron-dense particles, whose locations were characterized within each profile. Peroxidase immunoreactive profiles were defined as having an electron density considerably greater than that seen in comparable structures in the surrounding neuropil. The neuronal classification of all the labeled profiles and their synaptic structure was determined by criteria from Peters and colleagues (Peters, 1991).

GluR1 immunogold particles were relatively sparse because of known tissue penetration impediment associated with post-embedding techniques (Adams et al., 2002). Thus, profiles containing one or more gold particles were considered to be selectively labeled. Although this method may result in an underestimation of receptor proteins within profiles of the neuropil, especially at postsynaptic membrane specializations (Adams et al., 2002; see Supplemental Figure 1), the use of pre-embedded immunogold particles allows for more precise visualization of labeled receptors than other techniques. To overcome this potential underestimation, the collective change from over 2,000 labeled profiles (see section 2.8.3 below) was used to examine cocaine-induced trafficking of GluR1-containing AMPARs. Further, this limitation would be expected to apply equally to both saline- and cocaine-treated tissue making comparisons of labeling appropriate. To ensure validity of this labeling criterion, the percentage of gold particles overlying myelin, which is not known to express AMPA GluR1 subunits thus serving as a control for spurious gold-silver deposits, was

examined. In our preparations less than 0.1% of observed gold particles were seen on myelin, whereas 92% of all immunogold particles were found in dendritic profiles within the neuropil.

The parameters used for between-group comparison include: locomotor activity, blood plasma cocaine levels, neuronal profile size, GluR1 immunogold distribution, and GluR1 synaptic analysis. A power analysis of the significant parametric tests was completed to evaluate confidence of the results and to ensure that adequate sample sizes were used. Significant findings had power values ranging from 0.681 to 0.997.

2.8.1 Locomotor Activity—The amount of time mice were ambulatory (in seconds) and the distance they traveled (in meters) were analyzed using a two factor mixed-design ANOVA with a between groups analysis of the drug effect on locomotor behavior and a repeated-measures analysis of drug injection trials (Days 1–12). A Students t-test was performed (on mice receiving cocaine only) to determine whether a significant change in locomotor activity exists between when mice initially receive cocaine (Day 1) and following repeated subcutaneous cocaine administration (Day 12). A Students t-test was also used to examine differences in locomotor activity on baseline measures and on Day 15 (cocaine withdrawal) as compared to saline-treated animals.

2.8.2 Cocaine Plasma levels—The differences in plasma levels of cocaine, and its metabolite benzoylecgonine, measured either 30 minutes or 72 hours after the final injection of cocaine were analyzed using a Students t-test.

2.8.3 Profile Size—The profile area, perimeter, average diameter, and form factor were measured for each profile [total of 2,394 profiles (2,200 dendrites); 1,221 in the parabrachial VTA and 1,173 in the paranigral VTA] using Microcomputer Image Device software (MCID; Imaging Research, St. Catharines, Ontario). A cluster analysis was performed to statistically sort the profiles by size and an ANOVA was conducted to evaluate size differences between the PB and PN VTA regions.

2.8.4 GluR1 Immunogold Labeling—GluR1 gold particles within dendritic profiles were characterized with respect to whether they contacted the plasma membrane, post-synaptic density, or cytoplasmic endomembranes. The number of particles at these locations were combined with size measurements of the profile obtained from the MCID program to give the following measures of gold particle density: the number of GluR1 gold particles on the plasma membrane divided by the perimeter of the profile (PM/perim), the cytoplasmic gold particles divided by the area of the profile (cyt/area), and the total number of gold particles divided by the area of the profile (total/area). The data for each neuronal population (either TH or non-TH containing dendrites) were analyzed separately using a two factor (drug treatment group X dendritic size) ANOVA followed by post-hoc analysis using a Tukey test.

2.8.5 GluR1 Synaptic Analysis—Synaptic labeling was examined by quantifying the ratio of GluR1-labeled synapses out of the total number of synapses per experimental group. In addition, the number of GluR1 gold particles at post-synaptic densities was counted. The ratio of synaptic labeling was evaluated using Chi-Squared analyses and differences in the number of synaptic GluR1 gold particles were analyzed with a single factor ANOVA.

3. Results

3.1 Persistent increase in locomotor activity with repeated cocaine administration

There were no significant difference in baseline measures of locomotor activity between mice that were randomly assigned to the different treatment group in either the time they were ambulatory ($t(28) = 0.25$, $p > 0.05$) or distance traveled ($t(28) = 0.11$, $p > 0.05$; Figure 2). However, after the first injection on Day 1, mice receiving cocaine showed a significant increase in locomotor activity compared to saline controls in both the amount of time they were ambulatory ($F(1, 29) = 9.84$, $p < 0.05$) and the distance they traveled ($F(1, 29) = 14.66$, $p < 0.05$). With each successive injection of cocaine (Days 1–12), there were incremental increases in both the ambulatory time ($F(5, 140) = 5.57$, $p < 0.05$) and the distance traveled ($F(5, 140) = 5.98$, $p < 0.05$). This suggests the development of locomotor sensitization to a static, but repeated, dose of cocaine. The increased locomotor activation persisted, even after a week of no cocaine administration, as revealed by a challenge dose of cocaine on Day 12 when mice receiving cocaine still had significantly greater ambulatory times ($t(28) = 4.97$, $p < 0.05$) and travel distances ($t(28) = 2.15$, $p < 0.05$) than the same measures taken after the first injection of cocaine (Day 1). Further, mice receiving cocaine continued to show increased locomotor activity, as compared to saline controls, days after cessation of cocaine administration. On Day 15, mice that had prior exposure to cocaine had significantly greater ambulatory times ($t(12) = 3.21$, $p < 0.05$) and travel distances ($t(12) = 2.92$, $p < 0.05$), in the absence of cocaine, than saline control animals. Indeed, locomotor activity on Day 15 in cocaine-treated animals was similar to that seen on the first day of cocaine administration even though no drug was given (time ambulatory: $t(28) = 0.76$, $p > 0.05$; distance traveled: $t(28) = 0.26$, $p > 0.05$). The findings demonstrate a perseverance of cocaine-induced locomotor sensitization even after cessation of cocaine administration suggesting a relatively permanent change in the function of the locomotor system following repeated exposure to cocaine.

3.2 Systemic presence of cocaine at 30 minutes post-injection, but not 72 hours

Plasma samples from cocaine-treated animals were analyzed at 30 minutes or 72 hours after the final injection to evaluate the systemic presence of cocaine at these time points. At 30 minutes after the final injection, plasma levels of cocaine and its metabolite, benzoylecgonine, were similar to those reported previously (Benuck et al., 1987; Reith et al., 1987; Figure 3). At 72 hours after the final cocaine injection neither compound was detected (cocaine: $t(5) = 4.73$, $p < 0.05$; benzoylecgonine: $t(5) = 6.26$, $p < 0.05$), demonstrating that cocaine is no longer systemically present or active 3 days after cessation of repeated cocaine administration.

3.3 Predominant GluR1 immunogold distribution in somatodendritic profiles of both dopamine and non-dopamine VTA neurons

The subcellular distribution of GluR1-containing AMPAR immunogold particles in the mouse VTA of the present study was similar to that previously reported in both the mouse (Lane et al., 2010) and rat VTA (Lane et al., 2008). GluR1 immunogold labeling was predominantly seen postsynaptically in dendrites and somata in both the PB and PN subregions of the VTA (Figure 4). The immunogold labeling was highly selective for individual dendrites and absent from other similar profiles within the same neuropil. Slightly more dendrites contained both TH peroxidase and GluR1 immunogold labeling as compared with those containing exclusively GluR1 immunogold particles. This is consistent with the abundance of dopamine neurons in the VTA (Swanson, 1982; Johnson and North, 1992).

In both single and dual labeled dendrites, GluR1 immunogold particles were primarily located within the cytoplasm (73%; $n = 2,155$) and associated with cytoplasmic

endomembranes (Figure 4). The remaining GluR1 immunogold particles (27%; $n = 815$) were in contact with the plasma membrane, most commonly in small and medium sized dendritic profiles, which are presumably more distal from the soma. Eighty-three percent of plasmalemmal GluR1 immunogold particles were seen at non-synaptic portions of the membrane, whereas only 17% were detected at post-synaptic densities (PSDs). Only asymmetric, excitatory-type synapses showed the PSD-associated GluR1 immunogold particles ($n = 135$; (Carlin et al., 1980; Rollenhagen and Lubke, 2006).

3.4 Regional differences in the size of GluR1-labeled dendrites

The majority of GluR1-labeled dendritic profiles, compiled of all treatment groups, were small to medium sized as measured by the average diameter of each transversely-cut dendrite. Small (ranging from 0.1 to 0.6 μm) and medium (ranging from 0.6 to 1.0 μm) dendrites each composed 45% of the total number of labeled dendritic profiles sampled. The remaining 10% of these dendritic profiles had an average diameter greater than 1.0 μm . These large dendritic profiles were the most variable in size (ranging from 1.0 – 2.49 μm), and may have included portions of the labeled somata.

Examination of tissue from control animals only, revealed differences in dendritic size (using the average cross-sectional diameters of dendritic profiles as the size measure) based on the type of dendritic profile and VTA region. Collectively, dendrites found within the PB VTA were slightly smaller than dendrites found in the PN VTA. In both VTA regions, however, dendrites that were not immunolabeled for TH were smaller than those that contained the TH immunoperoxidase reaction product ($F(3,619) = 3.14$, $p < 0.05$; PB VTA: TH dendrites: $0.69 \mu\text{m} \pm .01$, non-TH dendrites: 0.57 ± 0.02 ; PN VTA: TH dendrites: 0.70 ± 0.02 , non-TH dendrites: $0.64 \mu\text{m} \pm 0.03$).

3.5 Increased synaptic GluR1 immunogold labeling in VTA dopamine dendrites reflects absence, not presence, of cocaine following chronic administration

3.5.1 - 30 minutes after repeated cocaine administration—TH-labeled dendrites within the VTA showed little difference, from saline controls, in subcellular GluR1 immunolabeling 30 minutes after repeated cocaine administration, despite the systemic presence of cocaine (Table 1). There was no significant change in either the number of GluR1-immunogold particles seen at the post-synaptic density (PB VTA: $F(2, 24) = 0.78$; PN VTA: $F(2, 37) = 1.48$, $p > 0.05$) or in the percentage of GluR1 immunogold labeled synapses (PB VTA: $\chi^2(1) = 1.00$; PN VTA: $\chi^2(1) = 1.18$, $p > 0.05$) in TH-containing dendrites of either VTA region at 30 minutes following repeated cocaine administration (Figure 5). Further at this time-point, post-hoc tests revealed no significant differences between cocaine-treated mice and saline controls in plasmalemmal GluR1 immunogold labeling in either VTA subregion. However, TH-immunoreactive dendrites of the PB VTA from mice receiving repeated cocaine injections did show a significant decrease in cytoplasmic ($F(2, 558) = 4.80$, $p < 0.05$) and total ($F(2, 558) = 4.37$, $p < 0.05$) GluR1 immunogold density. The opposite was evident in the PN VTA where there was a slight increase in cytoplasmic GluR1 immunogold density in small TH-labeled dendrites, however this change was not statistically significant ($F(2, 311) = 2.47$, $p > 0.05$).

3.5.2 - 72 hours after repeated cocaine administration—Cessation of cocaine administration for 72 hours resulted in a significant increase in the number of synapses labeled with GluR1 immunogold particles in TH-immunoreactive dendrites of the PB VTA ($\chi^2(2) = 25.07$, $p < 0.05$), but did not affect the number of GluR1 immunogold particles at each synapse ($F(2, 39) = 1.08$, $p > 0.05$), as compared to saline controls. There was also a significant increase in plasmalemmal ($F(2, 558) = 3.81$, $p < 0.05$) with a concurrent decrease in cytoplasmic ($F(2, 558) = 4.80$, $p < 0.05$) and total ($F(2, 558) = 4.37$, $p < 0.05$) GluR1 immunogold density.

GluR1 immunogold density in TH-containing dendrites of the PB VTA (Figure 5). This suggests trafficking of GluR1-containing AMPARs from internal stores within the cytoplasm to the plasma membrane, including synaptic densities, at 72 hours after repeated cocaine administration.

In TH-labeled dendrites within the PN VTA, absence of cocaine following repeated administrations also resulted in a significant increase in the number of synapses labeled with GluR1 immunogold particles ($\chi^2(2) = 25.07$, $p < 0.05$), with no change in the number of gold particles at each synapse ($F(2,37) = 1.48$, $p > 0.05$), as compared to saline controls. However, in contrast to the PB VTA, TH-labeled dendrites of the PN VTA showed a significant decrease in plasmalemmal ($F(2, 238) = 3.48$, $p < 0.05$) GluR1 immunogold density, with no changes in cytoplasmic ($F(2, 238) = 2.48$, $p > 0.05$) or total densities of these particles ($F(2, 238) = 0.83$, $p > 0.05$) following 72 hours of abstinence from repeated cocaine administration. This suggests that in the absence of cocaine, the synaptic insertion of GluR1-containing AMPARs comes from lateral movement of these receptors along the plasma membrane. The findings demonstrate VTA region-specific synaptic trafficking mechanisms of GluR1-containing AMPARs in dopaminergic neurons when cocaine is depleted following repeated administrations.

3.6 Repeated cocaine administration increases surface GluR1 immunogold labeling in non-dopamine dendrites, an effect that persists in the absence of cocaine

3.6.1 - 30 minutes after repeated cocaine administration—Thirty minutes after the final of 6 repeated cocaine injections, there was a differential region-specific change in the subcellular distribution of AMPAR GluR1 subunits in non-TH containing dendrites of the VTA. Within the PB VTA, non-TH containing dendrites showed a significant increase in the plasmalemmal ($F(2, 115) = 3.06$, $p < 0.05$) with a concurrent decrease in cytoplasmic ($F(2, 115) = 3.35$, $p < 0.05$) GluR1 immunogold density suggesting surface trafficking of GluR1-containing AMPARs (Figure 6). However, there were no significant differences, as compared to saline controls, in either the number of gold particles at synapses ($F(2, 39) = 1.08$, $p > 0.05$) or in the proportion of labeled synapses ($\chi^2(2) = 5.14$, $p > 0.05$) at 30 minutes after repeated cocaine administration. In the PN VTA, repeated cocaine administration resulted in a significant increase in the number of total synapses labeled with GluR1 immunogold particles ($\chi^2(2) = 20.53$, $p < 0.05$), but no difference in the number of gold particles at each synapse ($F(2, 18) = 0.65$, $p > 0.05$) in non-TH containing dendrites. Further, there were no statistically significant changes in the plasmalemmal ($F(2, 332) = 0.48$, $p > 0.05$) or cytoplasmic ($F(2, 332) = 0.14$, $p > 0.05$) GluR1 immunogold density, as compared to saline controls. There were no significant changes in total GluR1 immunogold density in non-TH containing dendrites of either region following repeated cocaine administration (PB VTA: $F(2, 311) = 0.86$, $p > 0.05$; PN VTA: $G(2, 332) = 0.03$, $p > 0.05$). There was a slight decrease in plasmalemmal GluR1 immunogold density suggesting that increased synaptic GluR1 immunogold labeling may be a function of lateral movement of GluR1-containing AMPA receptor along the plasma membrane to post-synaptic specializations. These findings provide anatomical evidence of differential repeated-cocaine induced plasticity, depending upon VTA region, both resulting in increased surface expression of GluR1-containing AMPA receptors potentially leading to increased activation of non-dopaminergic neurons in the presence of cocaine.

3.6.2 - 72 hours after repeated cocaine administration—Non-TH containing dendrites maintain similar patterns in the subcellular distribution of GluR1 immunogold particles at 72 hours as was seen at 30 minutes following repeated cocaine administration. In the PB VTA, there was a significant increase in plasmalemmal GluR1 immunogold density ($F(2, 115) = 3.06$, $p < 0.05$), but no significant change in synaptic GluR1 immunogold

labeling in the PB VTA ($\chi^2 (2) = 5.13$, $p > 0.05$; Figure 6). In non-TH containing dendrites of the PN VTA, there was a significant increase the number of GluR1 labeled synapses ($\chi^2 (2) = 20.53$, $p < 0.05$), but no change in plasmalemmal, cytoplasmic, or total GluR1 immunogold density, as compared to saline controls.

3.7 Increases in synaptic GluR1 are associated with region-specific decreases in multivesicular bodies (MVBs)

The observed increases in synaptic GluR1 at 72 hours after repeated cocaine administration was associated with a significant decrease in the number of multivesicular bodies (MVBs) seen in TH-labeled dendrites of the PB VTA, as compared to saline controls ($\chi^2 (2) = 6.73$, $p < 0.05$), a change that was not evident at 30 minutes after repeated cocaine administration (Figure 7). TH-containing dendrites of the PN VTA also had increased synaptic GluR1 labeling at 72 hours following cocaine cessation. However, these dendrites showed no significant change in the number of MVBs, as compared to saline controls, at either 30 minutes or 72 hours after repeated cocaine administration ($\chi^2 (2) = 3.79$, $p > 0.05$). This suggests MVBs may play a role in the observed regional differences in AMPAR synaptic trafficking mechanisms (see 3.5.2 above). A decrease in the number of MVBs was also seen in non-TH containing dendrites of the PN VTA at 72 hours following repeated cocaine, although this effect was not statistically significant ($\chi^2 (2) = 5.64$, $p > 0.05$). Additionally, there were no significant changes in the number of MVBs in non-TH labeled dendrites of the PB VTA ($\chi^2 (2) = 0.17$, $p > 0.05$).

4. Discussion

Our results provide the first ultrastructural evidence that surface AMPA GluR1 trafficking, consistent with increased excitability, occurs preferentially in non-dopaminergic neurons of the VTA when in the presence of systemic cocaine following repeated administrations. A theoretical model is proposed in which repeated cocaine administration leads to a progressive inhibition of VTA dopamine neurons, and possibly other afferent targets of these cells including the nucleus accumbens (NAc; Carr and Sesack, 2000a; Carr and Sesack, 2000b; Van Bockstaele and Pickel, 1995; Yim and Mogenson, 1980), while cocaine is systemically present. Conceivably, the heightened activity of GABAergic neurons, while in the presence of cocaine, may reflect a homeostatic mechanism meant to offset the stimulant effects of cocaine. In contrast, when systemic cocaine is depleted we show further increases in synaptic GluR1 trafficking in non-dopamine neurons of the PN VTA and in dopamine neurons throughout the VTA. This suggests that the absence, not the presence of cocaine, following repeated administrations, elicits the increased glutamatergic activation of VTA dopamine neurons, a proposed mechanism for the development of locomotor sensitization and addiction to cocaine (Carlezon and Nestler, 2002; Kalivas and Stewart, 1991; Kessels and Malinow, 2009; Wolf, 2003). The observed AMPAR GluR1 trafficking in non-dopamine and dopamine neurons of the VTA in the respective presence or absence of cocaine is schematically diagramed in Figure 8.

4.1 Preferential increase in synaptic and plasmalemmal GluR1 immunogold labeling in non-dopaminergic dendrites in the presence of systemic cocaine

At 30 minutes following repeated cocaine administration, dopaminergic dendrites of both VTA regions have similar subcellular distributions of AMPAR GluR1 subunits as those seen in cocaine naïve animals. The comparable GluR1 distributions occur despite our finding of the presence of systemic cocaine at this time point, which is consistent with prior reports of cocaine plasma concentrations (Benuck et al., 1987; Pan and Hedaya, 1998). The lack of notable changes in subcellular GluR1 distributions while in the presence of cocaine differs from that seen at 30 minutes following a single injection of the same cocaine dose (Lane et

al., 2010). Together, the data suggest a loss of acute cocaine-induced plasticity of GluR1-containing AMPARs in VTA dopaminergic neurons with repeated administration.

In contrast with TH-labeled dendrites, non-TH containing dendrites show increased numbers of synapses with AMPAR GluR1 immunogold labeling in the PN VTA and increased plasmalemmal AMPAR GluR1 immunogold density in the PB VTA. The non-TH containing dendrites are most likely GABAergic, the second most prevalent cell type within the VTA (Nagai et al., 1983; Steffensen et al., 1998). These cells act as interneurons, exerting tonic inhibition of dopaminergic neurons in the VTA (Xi and Stein, 1998; Yim and Mogenson, 1980). Increased AMPAR-mediated activation of these cells results in decreased dopamine release in the NAc (Kalivas and Duffy, 1990; Xi and Stein, 1998). As such, VTA GABA neurons may play an important role in reward and addiction by varying inhibitory control of dopaminergic neurons (Steffensen et al., 1998; Xi and Stein, 1998).

Alternately, increases in VTA GABA release may decrease cocaine-induced activation of dopamine neurons via GABA_B receptors (Backes and Hemby, 2007; Laviolette and Van Der Kooy, 2001). GABA_B receptors are found predominantly on dopaminergic neurons in the VTA (Laviolette and Van Der Kooy, 2001) and activation of these receptors has been shown to enhance metabotropic glutamate receptor (mGluR) function (Kelley et al., 2009). Metabotropic glutamate receptors regulate AMPA receptor trafficking and activation of both mGluRs and GABA_B receptors change AMPA receptor subunit composition leading to a reduction in the number of synaptic calcium-permeable AMPA receptors (Kelley et al., 2009). The composition of AMPA receptor subunits is important because GluR1/GluR3 heteromers or GluR1 homomers allow calcium permeability to the cell, whereas inclusion of the GluR2 subunit renders the receptor calcium impermeable (Hollmann et al., 1991, Cull-Candy et al., 2006). Insertion of GluR2-containing AMPAR at the synapse are important in reversing GluR1-mediated synaptic activation and leads to induction of long term depression (LTD) of VTA dopaminergic neurons (Bellone and Luscher, 2005; Mameli et al., 2007).

Similarly, changes in synaptic activity and/or strength have been shown to trigger glial-mediated modulation of AMPA receptor trafficking to balance excitation/inhibition during periods of intense change in synapse number or strength (Stellwagen et al., 2005; Stellwagen and Malenka, 2006; Gainey et al., 2009; Zhao et al., 2010). The large increase in synaptic GluR1 in VTA dopamine neurons that is produced by a single injection of cocaine (Lane et al., 2010) may be sufficient to trigger a glial response resulting in a rebound decrease in synaptic GluR1 as presently seen in VTA dopamine neurons with repeated cocaine administration. These adaptations, which govern AMPAR subunit composition, may lead to changes in the synaptic insertion or removal, depending upon VTA region, of GluR1-containing AMPA receptors. Together, our results suggest that repeated cocaine exposure may activate homeostatic mechanisms meant to offset acute cocaine-induced changes in GluR1-containing AMPAR activation of VTA dopaminergic neurons. These mechanisms may contribute to the apparent “normal” AMPA GluR1 subcellular distributions of these VTA dopamine neurons in the systemic presence of cocaine. Thus, both GABA- and glial-mediated homeostatic mechanisms, which govern AMPAR subunit composition, may contribute to cocaine-induced changes in the synaptic content of GluR1-containing AMPARs.

4.2 Increased synaptic GluR1 immunogold labeling in both dopaminergic and non-dopaminergic neurons in the absence of cocaine following repeated administration

Dopamine dendrites, in both the PB and PN VTA, have significantly more GluR1 immunogold labeled synapses after 72 hours of abstinence following repeated cocaine administration compared to saline controls. Since we detected no systemic cocaine at this time point, we conclude that depletion of cocaine, following repeated administration,

initiates synaptic mobilization of GluR1-containing AMPAR in VTA dopamine neurons. Synaptic insertion of GluR1-containing AMPARs is a necessary first step in the development of neuronal changes resulting in increased excitability of dopaminergic neurons (Argilli et al., 2008; Liao et al., 2001; Malinow and Malenka, 2002; Park et al., 2004). This activation may significantly contribute to the development of addiction to cocaine (Bellone and Luscher, 2006; Kalivas and Stewart, 1991; Kessels and Malinow, 2009; Wolf, 2003).

Our finding that repeated cocaine administration affects the subcellular AMPAR GluR1 distribution at 72 hours, but not 30 minutes, supports previous molecular and electrophysiological studies showing increased AMPAR GluR1 protein expression (Fitzgerald et al., 1996) and AMPA-mediated glutamatergic activation of VTA dopaminergic neurons (Bellone and Luscher, 2006; Borgland et al., 2004; Saal et al., 2003) after withdrawal (12–24 hours) from repeated cocaine administration. Thus, the absence of residual cocaine after prior exposure may trigger an increase in AMPAR-mediated activation of VTA dopaminergic neurons. This suggests that the withdrawal following intermittent chronic administration of cocaine is important for triggering neuroadaptive changes leading to addiction.

Moreover, at 72 hours after the final cocaine injection, there are significant increases in the number of GluR1 immunogold labeled synapses in non-TH labeled dendrites only in the PN VTA. GABA neurons in this VTA region receive direct glutamatergic input from the PFC (Carr and Sesack, 2000) and modulate the activity of VTA dopamine neurons (Steffensen et al., 1998; Xi and Stein, 1998). Thus, the potential added inhibition of VTA dopamine neurons, in the absence of cocaine, may explain anhedonia and withdrawal associated with cessation of chronic cocaine use (Covington and Miczek, 2003; Matsumoto and Hikosaka, 2009).

4.3 VTA region-specific differences in AMPAR GluR1 trafficking

Dopaminergic dendrites throughout the VTA show increased synaptic GluR1 labeling in the absence of cocaine, following repeated administration, however, the mechanism for this synaptic AMPAR insertion appears to differ by VTA region. In dopamine neurons of the PB VTA, a region having substantial mesocortical projections, the increase in synaptic GluR1, seen at 72 hours after the last of 6 cocaine injections, occurred together with increased plasmalemmal, but decreased cytoplasmic, GluR1 immunogold density and decreased numbers of MVBs. This suggests trafficking of internalized GluR1-containing AMPARs to both synaptic and non-synaptic sites of the plasma membrane. AMPAR are highly mobile and can move between intracellular compartments and the plasma membrane via exo- and endocytosis (Malinow and Malenka, 2002). Moreover, recycling endosomes, such as MVBs, are thought to influence receptor exo/endocytosis by regulating the amount of receptor availability (Ehlers, 2000; Luscher et al., 1999; Park et al., 2004).

In dopamine neurons of the PN VTA, a region enriched in mesolimbic projecting cells, the absence of cocaine following repeated administration resulted in an increase in synaptic GluR1 labeling. This increase in synaptic GluR1 labeling occurred in conjunction with a significant decrease in the plasmalemmal density of GluR1-containing AMPARs but no change in the cytoplasmic density of GluR1 immunogold labeling or in the number of MVBs, as compared to saline controls. This suggests that, in this region GluR1-containing AMPARs moved to synaptic sites laterally along the plasma membrane (Choquet and Triller, 2003; Malinow and Malenka, 2002). It is not clear why there are regional differences in AMPA receptor GluR1 synaptic trafficking, however, receptor exocytosis is a slower process taking minutes for synaptic receptor insertion, whereas insertion via lateral movement can occur within milliseconds (Adesnik et al., 2005). As such, the differences in

AMPA trafficking witnessed between the two VTA regions may reflect, in part, difference in the magnitude of glutamatergic activation of dopaminergic neurons in the two VTA regions (Ehlers, 2000; Lee et al., 2004).

4.4 Locomotor sensitization to repeated cocaine administration

Repeated cocaine administration resulted in a progressive increase in locomotor activity across injection trials demonstrating the development of sensitization to the stimulant effects of cocaine. Increased locomotor activity is attributed to enhanced AMPAR-mediated activation of VTA dopamine neurons (Borgland et al., 2004; Carlezon et al., 1997; Karler et al., 1989; Wolf, 1998). However, we observed no changes in the subcellular AMPAR GluR1 distribution in VTA dopaminergic neurons in the presence of systemic cocaine, at the time when locomotor activity was measured. In contrast with dopamine neurons, synaptic GluR1 labeling was increased in putative GABAergic neurons within the PN VTA, 30 minutes after cocaine administration, which would result in increased inhibition of mesolimbic dopamine neurons that extensively project to the NAc (Carr and Sesack, 2000b; Lane et al., 2008). Furthermore, VTA GABAergic neurons also project directly to the NAc (Omelchenko and Sesack, 2009) where enhanced inhibition of output neurons is related to cocaine-induced increases in locomotor activity (Henry and White, 1995).

The large increase in synaptic GluR1 observed in VTA dopamine neurons when cocaine is systemically depleted may be an important trigger for increasing locomotor activity. This idea is supported by our demonstration of increased locomotor activity on test day 15, despite receiving no cocaine, in mice that previously received repeated cocaine administration, as compared to cocaine naïve mice. This persistent enhancement in locomotor activity is consistent with the development of long term potentiation (LTP). Stimuli that induce LTP increase the number of functional AMPARs at the synapse, a necessary first step for NMDA receptor activation (Liao et al., 1995; Malinow and Malenka, 2002; Park et al., 2004) and NMDA-dependent LTP can be elicited from neurons within the VTA (Bonci and Malenka, 1999). Thus, the intermittent-repeated pattern of cocaine administration may trigger relatively permanent change in the function of VTA dopaminergic neurons resulting in the development of locomotor sensitization, a behavioral paradigm related to drug craving and addiction (Carlezon et al., 1997; Kalivas and Stewart, 1991; Robinson and Berridge, 1993).

5. Implications

Our findings provide new insight into the cellular mechanisms by which the repeated “on” and “off” stimulation of VTA dopaminergic neurons, associated with repeated intermittent cocaine use, may greatly strengthen the maladaptive neuroplasticity of the mesocorticolimbic dopaminergic system. Such plasticity is highly relevant to the progressively decreasing reward and increasing propensity for drug seeking and relapse following repeated cocaine administration. The intermittent exposure to cocaine produces a progressive and enduring increase in synaptic GluR1-containing AMPARs in non-dopamine, putative GABAergic, neurons in the presence of cocaine. These changes can lead to increased inhibition of mesolimbic dopamine neurons and potentially activate GABA-mediated homeostatic mechanisms. Together these inhibitory mechanisms may partially explain the observed decreasing reward associated with chronic cocaine use. In the absence of cocaine, however, the increased synaptic GluR1 seen in dopamine neurons of the VTA may enhance AMPAR-mediated activation of these cells, which is requisite for initiating long-lasting changes in function. Thus, the differential GluR1 plasticity witnessed in VTA neurons in the presence or absence of cocaine suggests that the repeated intermittency of cocaine delivery may be an important component in the development of addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants NIH-NIDA P60 05130 to MJK and VMP, NIH-NIDA R01 004600 to VMP, NIH-NIDA K05 00049 to MJK, and NIH-NIDA T32 007274 to CEI. We would like to thank Ms. Danielle Robinson for help in editing.

Abbreviations

VTA	Ventral Tegmental Area
PB VTA	Parabrachial Ventral Tegmental Area
PN VTA	Paranigral Ventral Tegmental Area
AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
DA	Dopamine
TH	Tyrosine Hydroxylase
GABA	γ -aminobutyric acid
MVB	multivesicular body
mPFC	Medial Prefrontal Cortex
NAc	Nucleus Accumbens
LTP	Long term potentiation
HPLC	High Performance Liquid Chromatography

References

- Adams MM, Fink SE, Shah RA, Janssen WG, Hayashi S, Milner TA, McEwen BS, Morrison JH. Estrogen and aging affect the subcellular distribution of estrogen receptor- α in the hippocampus of female rats. *J Neurosci.* 2002; 22:3608–14. [PubMed: 11978836]
- Adesnik H, Nicoll RA, England PM. Photoinactivation of native AMPA receptors reveals their real-time trafficking. *Neuron.* 2005; 48:977–85. [PubMed: 16364901]
- Aicher SA, Sharma S, Mitchell JL. Co-localization of AMPA receptor subunits in the nucleus of the solitary tract in the rat. *Brain Res.* 2002; 958:454–8. [PubMed: 12470884]
- Argilli E, Sibley DR, Malenka RC, England PM, Bonci A. Mechanism and time course of cocaine-induced long-term potentiation in the ventral tegmental area. *J Neurosci.* 2008; 28:9092–100. [PubMed: 18784289]
- Backes EN, Hemby SE. Contribution of ventral tegmental GABA receptors to cocaine self-administration in rats. *Neurochem Res.* 2008; 33:459–67. [PubMed: 17943439]
- Bayer VE, Pickel VM. GABA-labeled terminals form proportionally more synapses with dopaminergic neurons containing low densities of tyrosine hydroxylase-immunoreactivity in rat ventral tegmental area. *Brain Res.* 1991; 559:44–55. [PubMed: 1685938]
- Bellone C, Luscher C. Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. *Nat Neurosci.* 2006; 9:636–41. [PubMed: 16582902]
- Benuck M, Lajtha A, Reith ME. Pharmacokinetics of systemically administered cocaine and locomotor stimulation in mice. *J Pharmacol Exp Ther.* 1987; 243:144–9. [PubMed: 3668848]
- Bonci A, Malenka RC. Properties and plasticity of excitatory synapses on dopaminergic and GABAergic cells in the ventral tegmental area. *J Neurosci.* 1999; 19:3723–30. [PubMed: 10234004]

- Borgland SL, Malenka RC, Bonci A. Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area: electrophysiological and behavioral correlates in individual rats. *J Neurosci*. 2004; 24:7482–90. [PubMed: 15329395]
- Capriles N, Rodaros D, Sorge RE, Stewart J. A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl)*. 2003; 168:66–74. [PubMed: 12442201]
- Carlezon WA Jr, Boundy VA, Haile CN, Lane SB, Kalb RG, Neve RL, Nestler EJ. Sensitization to morphine induced by viral-mediated gene transfer. *Science*. 1997; 277:812–4. [PubMed: 9242609]
- Carlezon WA Jr, Nestler EJ. Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci*. 2002; 25:610–5. [PubMed: 12446127]
- Carlin RK, Grab DJ, Cohen RS, Siekevitz P. Isolation and characterization of postsynaptic densities from various brain regions: enrichment of different types of postsynaptic densities. *J Cell Biol*. 1980; 86:831–45. [PubMed: 7410481]
- Carr DB, Sesack SR. GABA-containing neurons in the rat ventral tegmental area project to the prefrontal cortex. *Synapse*. 2000a; 38:114–23. [PubMed: 11018785]
- Carr DB, Sesack SR. Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J Neurosci*. 2000b; 20:3864–73. [PubMed: 10804226]
- Chan J, Aoki C, Pickel VM. Optimization of differential immunogold-silver and peroxidase labeling with maintenance of ultrastructure in brain sections before plastic embedding. *J Neurosci Methods*. 1990; 33:113–27. [PubMed: 1977960]
- Choquet D, Triller A. The role of receptor diffusion in the organization of the postsynaptic membrane. *Nat Rev Neurosci*. 2003; 4:251–65. [PubMed: 12671642]
- Cornish JL, Kalivas PW. Repeated cocaine administration into the rat ventral tegmental area produces behavioral sensitization to a systemic cocaine challenge. *Behav Brain Res*. 2001; 126:205–9. [PubMed: 11704265]
- Covington HE III, Miczek KA. Vocalizations during withdrawal from opiates and cocaine: possible expressions of affective distress. *Eur J Pharm*. 2003; 467:1–13.
- Dunn JM, Inderwies BR, Licata SC, Pierce RC. Repeated administration of AMPA or a metabotropic glutamate receptor agonist into the rat ventral tegmental area augments the subsequent behavioral hyperactivity induced by cocaine. *Psychopharmacology (Berl)*. 2005; 179:172–80. [PubMed: 15580483]
- Ehlers MD. Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron*. 2000; 28:511–25. [PubMed: 11144360]
- Fitzgerald LW, Ortiz J, Hamedani AG, Nestler EJ. Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. *J Neurosci*. 1996; 16:274–82. [PubMed: 8613793]
- Gainey MA, Hurvitz-Wolff JR, Lambo ME, Turrigiano GG. Synaptic scaling requires the GluR2 subunit of the AMPA receptor. *J Neurosci*. 2009; 29:6479–89. [PubMed: 19458219]
- Glass MJ, Kruzich PJ, Colago EE, Kreek MJ, Pickel VM. Increased AMPA GluR1 receptor subunit labeling on the plasma membrane of dendrites in the basolateral amygdala of rats self-administering morphine. *Synapse*. 2005; 58:1–12. [PubMed: 16037950]
- Goeders NE, Smith JE. Cortical dopaminergic involvement in cocaine reinforcement. *Science*. 1983; 221:773–5. [PubMed: 6879176]
- Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature*. 2001; 412:141–2. [PubMed: 11449260]
- Harris GC, Aston-Jones G. Critical role for ventral tegmental glutamate in preference for a cocaine-conditioned environment. *Neuropsychopharmacology*. 2003; 28:73–6. [PubMed: 12496942]
- Henry DJ, White FJ. The persistence of behavioral sensitization to cocaine parallels enhanced inhibition of nucleus accumbens neurons. *J Neurosci*. 1995; 15:6287–99. [PubMed: 7666211]
- Hof, PR.; Young, WG.; Bloom, FE.; Belinchenko, PV.; Celio, MR., editors. *Comparative cytoarchitectonic atlas of the C57BL/6 and 129/Sv mouse brains*. Amsterdam: Elsevier; 2000.

- Jentsch JD, Taylor JR. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl)*. 1999; 146:373–90. [PubMed: 10550488]
- Johnson SW, North RA. Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *J Physiol*. 1992; 450:455–68. [PubMed: 1331427]
- Kalivas PW, Duffy P. Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release. *J Neurochem*. 1988; 50:1498–504. [PubMed: 3361305]
- Kalivas PW, Duffy P. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse*. 1990; 5:48–58. [PubMed: 2300906]
- Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev*. 1991; 16:223–44. [PubMed: 1665095]
- Karler R, Calder LD, Chaudhry IA, Turkanis SA. Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. *Life Sci*. 1989; 45:599–606. [PubMed: 2671566]
- Kauer JA, Malenka RC. Synaptic plasticity and addiction. *Nat Rev Neurosci*. 2007; 8:844–58. [PubMed: 17948030]
- Kelly L, Farrant M, Cull-Candy SG. Synaptic mGluR activation drives plasticity of calcium-permeable AMPA receptors. *Nat Neurosci*. 2009; 12:593–601. [PubMed: 19377472]
- Kessels HW, Malinow R. Synaptic AMPA receptor plasticity and behavior. *Neuron*. 2009; 61:340–50. [PubMed: 19217372]
- Lane DA, Jaferi A, Kreek MJ, Pickel VM. Acute and chronic cocaine differentially alter the subcellular distribution of AMPA GluR1 subunits in region-specific neurons within the mouse ventral tegmental area. *Neuroscience*. 2010; 169:559–73. [PubMed: 20553819]
- Lane DA, Lessard AA, Chan J, Colago EE, Zhou Y, Schlussman SD, Kreek MJ, Pickel VM. Region-specific changes in the subcellular distribution of AMPA receptor GluR1 subunit in the rat ventral tegmental area after acute or chronic morphine administration. *J Neurosci*. 2008; 28:9670–81. [PubMed: 18815253]
- Laviolette SR, van der Kooy D. GABA(A) receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems. *Eur J Neurosci*. 2001; 13:1009–15. [PubMed: 11264674]
- Lee SH, Simonetta A, Sheng M. Subunit rules governing the sorting of internalized AMPA receptors in hippocampal neurons. *Neuron*. 2004; 43:221–36. [PubMed: 15260958]
- Leranth, CaPVM. Electron Microscopic Pre-embedding Double Immunostaining Methods. In: LaZ; Heimer, L., editors. *Tract Tracing Methods II: Recent Progress*. Plenum; New York: 1989.
- Lessard A, Coleman CG, Pickel VM. Chronic intermittent hypoxia reduces neurokinin-1 (NK(1)) receptor density in small dendrites of non-catecholaminergic neurons in mouse nucleus tractus solitarius. *Exp Neurol*. 2006; 223:634–44. [PubMed: 20206166]
- Liao D, Hessler NA, Malinow R. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature*. 1995; 375:400–4. [PubMed: 7760933]
- Liao D, Scannevin RH, Haganir R. Activation of silent synapses by rapid activity-dependent synaptic recruitment of AMPA receptors. *J Neurosci*. 2001; 21:6008–17. [PubMed: 11487624]
- Lu L, Grimm JW, Dempsey J, Shaham Y. Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. *Psychopharmacology (Berl)*. 2004; 176:101–8. [PubMed: 15071719]
- Luscher C, Xia H, Beattie EC, Carroll RC, von Zastrow M, Malenka RC, Nicoll RA. Role of AMPA receptor cycling in synaptic transmission and plasticity. *Neuron*. 1999; 24:649–58. [PubMed: 10595516]
- Malinow R, Malenka RC. AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci*. 2002; 25:103–26. [PubMed: 12052905]
- Mameli M, Bolland B, Lujan R, Luscher C. Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. *Science*. 2007; 317:530–3. [PubMed: 17656725]
- Matsumoto M, Hikosaka O. Two types of dopamine neurons distinctly convey positive and negative motivational signals. *Nature*. 2009; 459:837–842. [PubMed: 19448610]

- McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci*. 2001; 21:8655–63. [PubMed: 11606653]
- Nagai T, McGreer PL, McGreer EG. Distribution of GABA-T-intensive neurons in the rat forebrain and midbrain. *J Comp Neurol*. 1983; 218:220–238. [PubMed: 6886073]
- Olson VG, Nestler EJ. Topographical organization of GABAergic neurons within the ventral tegmental area of the rat. *Synapse*. 2007; 61:87–95. [PubMed: 17117419]
- Omelchenko N, Sesack SR. Ultrastructural analysis of local collaterals of rat ventral tegmental area neurons: GABA phenotype and synapses onto dopamine and GABA cells. *Synapse*. 2009; 63:895–906. [PubMed: 19582784]
- Pan WJ, Hedaya MA. An animal model for simultaneous pharmacokinetic/pharmacodynamic investigations: application to cocaine. *J Pharmacol Toxicol Methods*. 1998; 39:1–8. [PubMed: 9596142]
- Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD. Recycling endosomes supply AMPA receptors for LTP. *Science*. 2004; 305:1972–5. [PubMed: 15448273]
- Park WK, Bari AA, Jey AR, Anderson SM, Spealman RD, Rowlett JK, Pierce RC. Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *J Neurosci*. 2002; 22:2916–25. [PubMed: 11923456]
- Peters, A.; Palay, SL.; Webster, HD. *The Fine Structure of the Nervous System*. Oxford University Press; New York: 1991.
- Reith ME, Benuck M, Lajtha A. Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. *J Pharmacol Exp Ther*. 1987; 243:281–7. [PubMed: 3668859]
- Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol*. 1963; 17:208–12. [PubMed: 13986422]
- Robbins TW. Dissociating executive functions of the prefrontal cortex. *Philos Trans R Soc Lond B Biol Sci*. 1996; 351:1463–70. discussion 1470–1. [PubMed: 8941958]
- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev*. 1993; 18:247–91. [PubMed: 8401595]
- Rollenhagen A, Lubke JH. The morphology of excitatory central synapses: from structure to function. *Cell Tissue Res*. 2006; 326:221–37. [PubMed: 16932936]
- Rusnak M, Gainer H. Differential effects of forskolin on tyrosine hydroxylase gene transcription in identified brainstem catecholaminergic neuronal subtypes in organotypic culture. *Eur J Neurosci*. 2005; 21:889–98. [PubMed: 15787695]
- Saal D, Dong Y, Bonci A, Malenka RC. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron*. 2003; 37:577–82. [PubMed: 12597856]
- Saal D, Dong Y, Bonci A, Malenka RC. Drugs of Abuse and Stress Trigger a Common Synaptic Adaptation in Dopamine Neurons. *Neuron*. 2003; 37:577–582. [PubMed: 12597856]
- Siegel SJ, Janssen WG, Tullai JW, Rogers SW, Moran T, Heinemann SF, Morrison JH. Distribution of the excitatory amino acid receptor subunits GluR2(4) in monkey hippocampus and colocalization with subunits GluR5–7 and NMDAR1. *J Neurosci*. 1995; 15:2707–19. [PubMed: 7722624]
- Steffensen SC, Svingos AL, Pickel VM, Henriksen SJ. Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *J Neurosci*. 1998; 18:8003–15. [PubMed: 9742167]
- Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor- α . *J Neurosci*. 2005; 25:3219–28. [PubMed: 15788779]
- Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF- α . *Nature*. 2006; 440:1054–9. [PubMed: 16547515]
- Sun W, Akins CK, Mattingly AE, Rebec GV. Ionotropic glutamate receptors in the ventral tegmental area regulate cocaine-seeking behavior in rats. *Neuropsychopharmacology*. 2005; 30:2073–81. [PubMed: 15841101]

- Swanson LW. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bul.* 1982; 9:321–353.
- Tang W, Wesley M, Freeman WM, Liang B, Hemby SE. Alterations in ionotropic glutamate receptor subunits during binge cocaine self-administration and withdrawal in rats. *J Neurochem.* 2004; 89:1021–33. [PubMed: 15140200]
- Ungless MA, Whistler JL, Malenka RC, Bonci A. Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature.* 2001; 411:583–7. [PubMed: 11385572]
- Van Bockstaele EJ, Pickel VM. GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res.* 1995; 682:215–21. [PubMed: 7552315]
- White FJ, Hu XT, Zhang XF, Wolf ME. Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. *J Pharmacol Exp Ther.* 1995; 273:445–54. [PubMed: 7714800]
- Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB Jr. Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. *Psychopharmacology (Berl).* 1995; 120:10–20. [PubMed: 7480530]
- Wolf ME. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog Neurobiol.* 1998; 54:679–720. [PubMed: 9560846]
- Wolf ME. LTP may trigger addiction. *Mol Interv.* 2003; 3:248–52. [PubMed: 14993438]
- Xi ZX, Stein EA. Nucleus accumbens dopamine release modulation by mesolimbic GABAA receptors-an in vivo electrochemical study. *Brain Res.* 1998; 798:156–65. [PubMed: 9666112]
- Yim CY, Mogenson GJ. Electrophysiological studies of neurons in the ventral tegmental area of Tsai. *Brain Res.* 1980; 181:301–13. [PubMed: 7350968]
- Zhang XF, Hu XT, White FJ, Wolf ME. Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves AMPA receptors. *J Pharmacol Exp Ther.* 1997; 281:699–706. [PubMed: 9152375]
- Zhao P, Leonoudakis D, Abood ME, Beattie EC. Cannabinoid receptor activation reduces TNF α -induced surface localization of AMPAR-type glutamate receptors and excitotoxicity. *Neuropharmacology.* 58:551–8. [PubMed: 19654014]

Figure 1.

Timeline of experimental procedures. Mice received a single subcutaneous injection of cocaine (15 mg/kg) once per day on Days 1–5 and a challenge dose one week later on Day 12. BL = baseline locomotor activity measures; LT = locomotor testing; TP = tissue preparation for either plasma cocaine levels or electron microscopic immunocytochemistry.

Figure 2.

Cocaine induced changes in locomotor activity. Bar graphs of locomotor activity showing that a single injection of cocaine (Day 1) produces a significant increase in both ambulatory time (A) and travel distance (B) as compared to baseline locomotor activity measures (* = $p < 0.05$). A progressive increase in ambulatory time (A) and travel distance (B) is seen with each successive injection of cocaine (♦ = $p < 0.05$). This sensitization to the locomotor stimulant effects of cocaine persist after a week without cocaine (Day 12) and in the absence of cocaine altogether (Day 15; ★ = $p < 0.05$).

Figure 3. Bar graphs showing plasma concentrations of cocaine and its metabolite, benzoylecgonine, at 30 minutes (□) and 72 hours (■) following the final of six subcutaneous cocaine (15 mg/kg) injections.

Figure 4.

VTA tissue sampling and predominant dendritic GluR1 and TH distributions. (A) Light micrograph of plastic embedded coronal section of tissue (-3.40 mm from Bregma) showing the area used for electron microscopic analysis (trapezoid). The sample area contains both the PB and PN VTA. (B) A representative electron micrograph of the VTA showing GluR1 immunogold labeling in both dopamine (TH + GluR1 den) and non-dopamine (GluR1 den) dendrites. TH is identified by the dense immunoperoxidase reaction product. Within these dendrites, GluR1 immunogold particles are most evident in the cytoplasm (gray arrows), but are also seen on the plasma membrane (white arrow). GluR1 labeling is highly selective for individual dendritic segments and absent from other similar profiles within the same neuropil (ul den). Both the electron micrograph and quantitative analysis of labeled profiles shown in the bar graph (C) demonstrate the mainly post-synaptic (dendritic) distribution of GluR1 immunogold particles. PB VTA, parabrachial ventral tegmental area; PN VTA, paranigral ventral tegmental area, SN, substantia nigra; ML, medial lemniscus; TH den, dendrite with TH peroxidase; ul den, unlabeled dendrites; ul te, unlabeled axon terminal; scale bar = $500\ \mu\text{m}$ (A) and $500\ \text{nm}$ (B).

Figure 5.

Subcellular GluR1 localization in TH-positive dendrites of the PB and PN VTA. Electron micrographs (A–C; G–I) and quantitative analysis (D–F; J–L) of GluR1 immunogold distributions in dendrites showing immunoperoxidase labeling for TH in the PB (top) and PN (bottom) VTA. In both VTA regions, TH-containing dendrites (TH + GluR1 den) have a predominantly cytoplasmic (gray arrows), with some plasmalemmal (white arrow), distribution of GluR1-gold particles in saline controls and at the 30 minute time point after repeated cocaine administration. However, VTA regional differences in GluR1-gold distributions are evident in TH-containing dendrites at 72 hours following repeated cocaine administration. In the PB VTA (C), increased synaptic (white triangle; D) and plasmalemmal (white arrow; E) GluR1 labeling is evident with a significant decrease in cytoplasmic (F) GluR1 density. In the PN VTA (I), increased synaptic (white triangles; J) GluR1 labeling is associated with a significant decrease in plasmalemmal (K), but no change in cytoplasmic (L) GluR1 immunogold density. GluR1 te, GluR1-immunogold labeled axon terminal; ul te, unlabeled axon terminal; curved black arrows = synapse; * $p < 0.05$; scale bar = 500 nm.

Parabrachial VTA: **Paranigral VTA:** **Figure 6.**

Subcellular GluR1 localization in non-TH dendrites of the PB and PN VTA. Electron micrographs (A–C; G–I) and quantitative analysis (D–F; J–L) of GluR1 immunogold distributions in dendrites containing GluR1 immunogold particles in the PB (top) and PN (bottom) VTA. In both VTA regions, these dendrites (GluR1 den) have a predominantly cytoplasmic (gray arrows) distribution of GluR1-gold particles in saline controls (A, G). In the PB VTA (top), at both the 30 min. and 72 hour time points following repeated cocaine administration (B, C), there is a significant increase in the GluR1 immunogold particle density seen at the plasma membrane (white arrows, E), with a concurrent decrease in cytoplasmic GluR1 immunogold density (F). In the PN VTA (bottom), increased synaptic GluR1 immunogold labeling (white triangles) was evident 30 min. after repeated cocaine administration (H), an effect that is amplified at 72 hrs (I). ul te, unlabeled axon terminal; curved black arrows = synapse; * $p < 0.05$; scale bar = 500 nm.

Figure 7.

Electron micrographs showing examples of MVBs in both TH- (A–B) and non TH-containing (C) dendrites within the VTA. MVBs are often seen near synaptic contacts (black arrow) that contain GluR1 immunogold particles (white triangles). Quantitative analysis (D–E) reveals a significant decrease in the number of MVBs seen in TH-positive dendrites of the PB VTA 72 hours after repeated cocaine administration. A similar decrease is seen at 72 hours after repeated cocaine administration in non-TH dendrites of the PN VTA, however this decrease is not statistically significant. MVB, multivesicular body; TH + GluR1 den, dopamine dendrite containing GluR1 immunogold particles; GluR1 den, dendrite containing GluR1 immunogold labeling; gray arrow, cytoplasmic GluR1 immunogold particle; ul te, unlabeled terminal; scale bar = 500 nm; * = $p < 0.05$.

Figure 8.

Schematic diagram showing basal distribution and cocaine-induced redistribution of GluR1 in subpopulations of dendrites in both the PB and PN VTA. (A) Basal distribution of AMPAR GluR1 in an excised cross-section of a small dendrite emergent from a VTA somata. The predominant cytoplasmic distribution is equally applicable to all dendrites, regardless of transmitter phenotype or VTA region. Some GluR1 gold particles (black circles) are also seen at the plasma membrane or at post-synaptic densities (PSD; darkened portion of the plasma membrane). GluR1 immunogold particles are often associated with small circular endomembranes (em) with the cytoplasm. (B) At 30 min. after repeated cocaine administration, a time when cocaine is systemically present, changes in AMPAR GluR1 immunogold distributions are most evident in non-DA dendrites. Repeated cocaine results in the trafficking of GluR1-containing AMPAR to the plasma membrane in the PB VTA, and to excitatory-type synapses in the PN VTA, possibly via lateral movement along

the plasma membrane. (C) At 72 hrs. after repeated cocaine administration, a time when cocaine is systemically absent, increased synaptic labeling of GluR1-containing AMPA receptors is evident in dopaminergic dendrites within both VTA regions and continued increased synaptic labeling is evident in nonDA neurons of the PN VTA (not shown). In the PB VTA, increased synaptic GluR1 occurs with a significant decrease in cytoplasmic GluR1 density suggesting relocation of GluR1-containing AMPA receptors from internal stores. Conversely, in the PN VTA, the increase in synaptic GluR1 is associated with decreased plasmalemmal, but no change in cytoplasmic, GluR1 density suggesting that GluR1-containing AMPARs move along the plasma membrane to PSD specializations. Black circles, GluR1 immunogold particles; gray circles, new location of GluR1 immunogold particles; white circles, prior location of GluR1 immunogold particles; em, endomembrane.

Summary of findings for DA and non-DA dendrites of both VTA regions at 30 min. and 72 hrs. following repeated cocaine administration.

Table 1

		30 min. after rep. cocaine		72 hrs. after rep. cocaine	
		PB VTA	PN VTA	PB VTA	PN VTA
DA Dendrites	Synaptic GluR1	NC	NC	↑*	↑*
	Plasmalemmal GluR1	NC	NC	↑*	↓*
	Cytoplasmic GluR1	↓*	NC	↓*	NC
Non-DA Dendrites	Synaptic GluR1	NC	↑*	NC	↑*
	Plasmalemmal GluR1	↑*	NC	↑*	NC
	Cytoplasmic GluR1	↓*	NC	NC	NC

NC, no significant change; ↑, increase; ↓, decrease
* p < 0.05.